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The Impacts of Rock Climbing on the Selection of Roosts by Bats and the Influence of These Mammals on the Biodiversity and Nutrient Influx of Cliff-Face Ecosystems

Ashley Kay Wilson

UNIVERSITY OF NORTHERN COLORADO
Greeley, Colorado
The Graduate School

THE IMPACTS OF ROCK CLIMBING ON THE SELECTION OF ROOSTS BY BATS AND THE INFLUENCE OF THESE MAMMALS ON THE BIODIVERSITY AND NUTRIENT INFLUX OF CLIFF-FACE ECOSYSTEMS

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Ashley Kay Wilson

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Accepted by the Doctoral Committee

_____
Rick A. Adams, Ph.D., Research Advisor

_____
Mitchell E. McGlaughlin, Ph.D., Committee Member

_____
Scott B. Franklin, Ph.D., Committee Member

_____
Corina E. Brown, Ph.D., Faculty Representative

Date of Dissertation Defense _____ _____

Accepted by the Graduate School

_____
Cindy Wesley
Interim Associate Provost and Dean
The Graduate School and International Admissions

ABSTRACT

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Humans have an un-matched ability to alter the environment, which often has detrimental effects on other species, from tiny microbes to enormous plants and animals. Bats are the second-largest order (Chiroptera) of mammals with ca. 1,300 described species, many of which have declining populations due to human disturbances of ecosystems. Being the only mammals capable of flight, bats exploit numerous ecosystems and are known to roost in various habitats including caves, trees, mines, bridges, barns, etc. Although the roosting ecology of bats in ecosystems such as caves and forests has been well documented, no study has yet examined the relationship between bats and any cliff-face biodiversity to date. My dissertation focused on the impacts of rock-climbing on the activity of bats on Dinosaur Mountain on the City of Boulder Open Space and Mountain Parks. I quantified 1) the number of bat roosts, 2) the number of emerging bats, and 3) the species richness and biodiversity of roosting bats at nine different cliffs with variable levels of rock-climbing. I also used multivariate statistics (canonical correspondence analysis) to correlate climbing characteristics (e.g. use-level, number of routes, average route difficulty, seasonal closures, etc.) with measures of bat activity (e.g. bat species richness and the number of roosts, emerging bats, and foraging bats). I also examined the relationships among the presence of cliff-

roosting bats on the biodiversity of organisms living in the soils of cliff crevices, as well as the biodiversity of mesofauna (e.g. small insects, spiders, mites, etc.). Specifically, I examined the biodiversity of bacteria and fungi using DNA analysis, and the biodiversity of mesofauna through visual microscopy. In addition, I used water quality assessment methods, which can be easily replicated in the field, to determine the influence of bats on the influx of nitrogen and phosphorus into cliff crevices, two elements that are essential for the establishment of microbes and flora in any ecosystem.

By quantifying the frequency of rock climbing via time-lapse cameras, I found that cliff sites with zero rock climbing (compared to low and high rock climbing) had significantly greater bat richness ($F_{2,105} = 7.25$, $p = 0.0011$), biodiversity ($F_{2,35} = 12.7$, $p < 0.0001$), number of roosting bats (per m^2) ($F_{2,105} = 7.25$, $p = 0.0011$), and number of foraging bats (per m^2) ($F_{2,105} = 8.67$, $p = 0.0003$). However, the number of roosts (per m^2) was significantly higher on cliffs with moderate levels of climbing ($F_{2,105} = 4.98$, $p = 0.009$). Through the DNA analysis, I found 400 species of bacteria and 269 species of fungi in the soils from the cliff crevices on Dinosaur Mountain. Overall, the biodiversity of bacteria was significantly higher in bat roosts compared to crevices without bats ($t = 2.33$, $p = 0.012$); although the biodiversity of fungi was slightly lower in crevices without bats, this difference was not significant ($t = 1.62$, $p = 0.056$). Bat guano contained significant levels of nitrate and phosphate, but no nitrite was detected. All three of these nutrients were higher in soils from/under bat roosts than in cliff crevices without bats ($H_2 \geq 7.82$, $p \leq 0.02$), suggesting that the presence of bat guano and urine increases nitrate and phosphate levels. Moreover, the increased bacterial biodiversity in bat roosts likely facilitates the higher levels of nitrite (as well as nitrate) through higher levels of

nitrification. Overall, rock-climbing appears to impact bats negatively, while bats seem to influence the biodiversity of soil bacteria and mesofauna positively, as well as increase the availability of usable nitrogen and phosphorus in cliff crevices, two nutrients that are essential for the development of all living organisms. The loss of bats on cliffs due to rock-climbing activities will likely have negative cascading effects on the biological communities of cliff faces, which are some of the most unique ecosystems on Earth.

DEDICATION

This dissertation is dedicated to my grandma Janelle, who passed away during the final stages of writing this paper. I love you, grandma, and I thank you for all the kindness, play-time, affection, and support you gave to me since I was a child. Even though we were not related by blood, you and I shared a bond that will always remain unmatched. Thank you for letting me be your granddaughter!

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INTRODUCTION

Most ecologists and conservation biologists accept the notion that we have entered a new epoch of geological time— the Anthropocene (Corlett 2015; McGill et al. 2015). Crutzen and Stoermer first coined this term in 2000, when they contended that humans have an unmatched ability to alter the ecosystems on Earth, particularly during the Industrial Revolution at the end of the 18th century (Crutzen and Stoermer 2000). With the rapid proliferation of the human population within just the last few centuries, many of the biophysical properties and natural processes of our planet have begun to deviate significantly from those of the Holocene (Corlett 2015). For example, the increased concentrations of greenhouse gases in Earth's atmosphere, the acidification of our oceans, and the homogenization of (once diverse) ecosystems, are all recent changes that have been tightly linked to human activities (Corlett 2015; Newbold et al. 2015). Furthermore, the removal and transportation of rare Earth minerals, and the increasing spread of invasive species by humans add to a multitude of factors contributing to elevated rates of species extinction, a primary characteristic of the Anthropocene (Dirzo et al. 2014). Although there is still debate on when the Holocene ended, and the Anthropocene began (Smith and Zeder 2013), many agree that this new epoch involves not only biological and atmospheric changes, but alterations to the geology of Earth as well. Thus, some suggest that the large-scale changes in the geology and abundance of radionuclides since the start of the atomic age in the 1950s, better marks the start of the

Anthropocene than do the events of late 18th century (Zalasiewicz et al. 2010). Regardless of when this new era began, today humans are undoubtedly and negatively affecting the geology and ecology of Earth.

It has been estimated that over 50% of the terrestrial land cover on Earth has been modified by humans (McGill et al. 2015), and the 7.5 billion+ persons on our planet are currently consuming more than 40% of its primary productivity (McGill et al. 2015). This leaves just 60% of the planet's resources for the survival of the remaining 10 – 15 million species on Earth. Vertebrate populations have decreased by an average of 52% over the last 40 years, with approximately one-quarter of mammalian species now being threatened or endangered (McGill et al. 2015). This rapid loss of irreplaceable fauna (and flora) has led to a decrease in global biodiversity, which has reached unprecedented levels (Newbold et al. 2015).

Many investigations have concluded that there is a direct link between biodiversity and ecosystem functions (e.g. primary productivity, nutrient cycling, nitrogen fixation, carbon sequestration, etc.— Tilman et al. 2012; Pasari et al. 2013), with many emergent properties of ecosystems being affected by the loss of just a single species within a community (Norris 2012). Furthermore, Tilman et al. (2012) examined the findings of 11 long-term studies that focused on the impacts of human-made alterations to the environment (i.e. fertilization, water availability, herbivory, fires, etc.), and found biodiversity to be the strongest driver of ecosystem function over time. Therefore, with the ever-increasing population of humankind (many of whom have little regard for their environmental impacts), documenting the biodiversity of our planet's ecosystems and understanding how anthropogenic factors influence the functions of these

habitats, is critical for the survival of the 8.7 million (eukaryotic) species living on Earth (Sweetlove 2011).

Cliff-Face Ecosystems

Since Arthur Tansley coined the term "ecosystem" in the early 19th century, biologists have carried out research in practically every ecosystem imaginable: grasslands, tundra, forests, deserts, oceans, wetlands (Larson et al. 2000), and even caves. Within cave ecosystems, bats have been found to be important conduits of energy and nutrients (Studier et al. 1991; Iskali and Zhang 2015), and populations of aquatic fauna (e.g. crustaceans) have been negatively impacted by the loss of bats within cave ecosystems (Hobbs and Bagley 1989). Despite the evidence that bats play critical roles in their underground, rocky ecosystems, very little research has been conducted on the faces of vertical cliffs, an ecosystem commonly exploited by bats. This gap in the literature is partially due to the difficulty of locating these ecosystems on aerial maps (Larson et al. 2000), in addition to the arduous nature of sampling at these types of locations. This was furthered by many members of the scientific community seeing cliffs as merely geologic formations, rather than unique ecosystems (Viles et al. 2008). However, Viles et al. (2008) argue that understanding the complex (and non-linear) relationships among ecological and geomorphological systems is critical for the proper management of rare ecosystems, such as the faces of vertical cliffs.

Despite the limited research conducted on cliffs, they are found on every continent (Larson et al. 2000). These ecosystems are heterogeneous in nature with various cracks and crevices that serve as different microhabitats within outcroppings of the rock, and cliffs composed of sedimentary rock have provide even greater habitat

heterogeneity (Larson et al. 2000). These microhabitats lead to variations in factors such as levels of moisture, exposure to wind, and ambient temperatures among the crevices of single cliff. The variability in microhabitats and microclimates seen on vertical cliffs can have major effects on the diversity of plants, animals, fungi, and bacteria, which can ultimately yield to relatively high levels of biodiversity in a small area of space (Larson et al. 2000). For example, cliffs in Jackson Co., Colorado support a unique assemblage of plants, containing both xeric and mesic species, suggesting that the heterogeneity of cliff faces offers numerous microhabitats for establishing a multitude of species (Graham and Knight 2004). Moreover, because vertical ecosystems are difficult to access by humans (and thus are relatively undisturbed), cliff faces have the potential to house rare and/or sensitive species not found elsewhere on Earth, and it has been hypothesized that the crevices of cliffs act as critical refugia for these rare organisms, by allowing them to avoid predators and competitors alike (Larson et al. 2000).

The Wildlife of Cliff-Face Ecosystems

As producers, plants are often dependent on a suite of abiotic factors such as levels of moisture, exposure to light, wind, and abundance of various nutrients (e.g. nitrogen, phosphorus, calcium, etc.— Larson 2000; Jobaggy and Jackon 2001); therefore, the extreme environmental conditions present on cliffs make it difficult for many species of plant to thrive (Larson et al. 1999). However, there appear to be a few genera and families (e.g. *Gingko biloba*, Cupressaceae) that are consistently found on cliffs throughout the world (Larson et al. 2000). The faces of most inland cliffs are characterized by sparse vegetation of lower ‘flora’ such as bryophytes, lichens, and algae, but many cliffs also have a high prevalence of perennial grasses, shrubs, and even trees

(Gerrath et al. 1995). For example, Graham and Knight (2004) identified 163 species of plant both on and near cliffs in Colorado, and 13 species of these plants were found only on the faces of the vertical cliffs. Of the species of woody plants found on both the faces and bases of cliffs, the individuals that are located on the faces of the cliffs typically grow more-slowly, and they are often stunted and deformed (Larson et al. 1999; Larson et al. 2000), alluding to differences in the nutrients available to plants rooted within the crevices of the cliffs.

In addition to flora, vertical cliffs around the world have been found to house a wide array of ectothermic fauna. Invertebrates in particular, play an important role in the breakdown of dead and decaying plant material for cliff-face ecosystems (Larson et al. 2000). Overall, the diversity of invertebrates on cliffs is relatively high; for example, Růžička and Zacharda (1994) left pitfalls traps open for ca. 1 year on cliffs in the Czech Republic, and found an abundance of Diptera, Aphidinea, Opiliones, and rhagidiid mites, in addition to 23 species of Araneae and 31 species of Coleoptera. Conversely, ectothermic vertebrates (e.g. amphibians and reptiles) are relatively uncommon to these extreme habitats. In the damp ecosystems of the Pacific Northwest, there are approximately 20 species of herpetofauna that use cliffs for protection from adverse weather, and in Oregon and Washington there are no fewer than eight species of amphibian and reptile that use cliff habitats solely for reproduction (Herrington 1988). Not only that, but in more-arid areas such as Utah, many species of reptile are well established at the top of buttes (Johnson 1986). Although most of the reptilian species located on the faces of cliffs are lizards (e.g. *Sceloperus undulatus, Cnemidophorus tigris, Urosaurus ornatus, Uta stansbriana*), the bull snake (*Pituophis catenifer*) is also

found on the faces of vertical cliffs, as this serpent has an uncommon ability to scale large rocks (Johnson 1986).

Cliff-face ecosystems are also capable of supporting a wide diversity of endothermic animals. For example, inland cliffs appear to be heavily dominated by raptors, such as members of Falconiformes and Strigiformes, which primarily use vertical cliffs for nesting (Janes 1985). Interestingly, it appears that avian richness is greater on cliff faces than surrounding ecosystems, which has been attributed to the variable topography, geomorphology, and microclimates of cliffs (Ward and Anderson 1988). It has been hypothesized that this habitat heterogeneity increases the variability of vegetation on cliff faces, ultimately minimizing the level of interspecific competition among birds in these rare ecosystems (Ward and Anderson 1988; Matheson and Larson 1998).

In addition to birds, cliffs are often associated with large mammals like bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), and mountain lions (*Puma concolor*); however, many smaller species of mammal often reside on cliffs for lodging, foraging, and/or reproduction. For example, numerous species of rodent (e.g. *Neotoma lepida, Rattus rattus, Mus musculus, Onchomys leucogaster*), as well as multiple species of insectivore (e.g. *Blarina brevicauda, Sorex fumeus*, *Clethrionomys gapperi*), have been documented to use cliff faces, and even rabbits (e.g. *Sylvilagus floridanus*, *Lepus americanus*) and striped skunks (*Mephitis mephitis*) have been observed on cliffs (Churcher and Fenton 1968; Churcher and Dodds 1979). Despite the fact that multiple species of mammal have been documented to use cliffs, arguably no

group of mammals are better at accessing and exploiting the crevices of vertical cliffs than the only volant mammal ever to evolve— bats.

Bats and Cliff-Face Ecosystems

Bats are the second largest order of mammals, with just over 1,300 species recognized (Tuttle 2016). Many of these species provide important ecosystem services such as pollinating plants (Bawa 1990), dispersing seeds (Flemming and Williams 1990), and regulating populations of noxious insects (Cleveland et al. 2006). Being the only mammals capable of powered flight, bats exploit essentially every terrestrial ecosystem on Earth, and many species often roost in caves, mines, and trees. Some species use man-made structures such as houses, bridges and barns for which the foraging ecology and roosting behaviors are well documented (Kunz and Lumsden 2003; Patterson et al. 2003). However, bats are also known to roost on the faces of vertical cliffs (Churcher and Fenton 1968), a terrestrial ecosystem that has only been limitedly studied, with previous research focusing primarily on plants.

Because bats feed on the wing, their guano likely supplies crevices with nutrients that are otherwise absent from cliff ecosystems, which may be essential for the growth and development of bacteria, fungi, and plants in these (often) nutrient-deprived cracks. For example, there are 17 essential elements for plant life, including calcium (constituent of cell walls and activates enzymes), magnesium (helps to maintain soil pH), and potassium (activates enzymes for the synthesis of carbohydrates and proteins); however, the macronutrients nitrogen (a major component of chlorophyll, amino acids, and nucleic acids) and phosphorus (needed to make ATP, a component of cell membranes and nucleic acids) are often the most-limiting factors for the growth of plants (Jobbagy and

Jackson 2001; Osman 2013). These elements have been detected in the guano of big brown bats (*Eptesicus fuscus*—Studier et al. 1991; Studier et al. 1994), a species that I have observed roosting on the rock-climbing cliffs in Boulder, CO; however, there has yet to be a study that compares levels of nutrients in different environments due to bats (i.e. by depositing bat guano). Although rainwater brings nutrients into terrestrial ecosystems, most cliff crevices are sheltered from precipitation. Therefore, the guano deposited into the crevices of cliffs by bats (while roosting), may be imperative for providing the essential nutrients to the soils of cliff-face ecosystems that are needed to sustain communities of plants and microorganisms (e.g. fungi and bacteria).

It has been found that avian guano provides essential nutrients that promote the establishment of lichens on cliff faces, and it has been suggested that the presence of birds on cliffs facilitates the growth of plant communities by introducing nitrogen into these ecosystems (Langevin 2015). Furthermore, the guano from birds has been found to influence the chemistry of soils, as well as the biodiversity of microbiota and arthropods within these substrates (Kolb et al. 2015). Not only that, but Iskali and Zhang (2015) suggest that Mexican free-tailed bats (*Tadarida brasiliensis*) play a critical role in the influx of nutrients and the biodiversity of invertebrates in Bracken Cave, by influencing the bottom-up dynamics of this cave ecosystem. Due to these findings, it is reasonable to assume that cliff-roosting bats also play a critical role in the nutritive dynamics of cliff-face ecosystems, by introducing guano into the crevices of cliffs.

The nutrients that bats likely bring into their cliff-crevice roosts may also be critical for the proliferation of bacteria and fungi within the soils of cliff crevices, as research has shown that rodent droppings provide an excellent source of nutrients for the

growth of microbes and arthropods (Cheeptham 2013). Furthermore, Ogórek et al. (2016) found the guano of bats to be a beneficial substrate for the growth and development of fungi, including a large proportion of *Penicillium* sp., a species of fungus known for its antibiotic properties. Because bacteria and fungi provide important ecosystems services (e.g. fixating nitrogen and decomposing detritus), bats may be the key to providing the necessary resources for these organisms to thrive in cliff crevices, thus promoting the overall health of cliff-face ecosystems.

High levels of bacterial biodiversity have been observed in caves, an ecosystem commonly exploited by bats for roosting. These caves are home to a large number of Actinobacteria, a phylum that contains bacterial species that produce approximately two-thirds of our natural antibiotics (Groth et al. 1999; Kieser et al. 2000). Because bats are known to roost in both caves and within the crevices of vertical cliffs, it is possible that the guano located in soils of cliff crevices (deposited by roosting bats) could facilitate the establishment of undiscovered species of bacteria from which new antibiotics could be isolated and used for fighting against drug-resistant pathogens.

The Formation of the Rocky Mountain Foothills

The Foothills of the southern Rocky Mountains are unique geological structures, the formation of which is still under debate. The rugged topography of this area consists of numerous flatiron formations, or large rocky outcroppings with a wide base and a steeply sloped face (ca. 45^{o}— Roach 2008) that narrows toward its summit. The oldest rocks of the southern Rocky Mountains consist of Precambrian metamorphic rock, which has become overlain with kilometers of limestone, dolomite, and sandstone (Roach 2008). These newer layers of substrate arose from when much of central North America

resided under a shallow sea during the Paleozoic era (Fan et al. 2014). It has been theorized that intense tectonic activity occurred ca. 35 – 80 million years ago, lead to a period of extreme mountain building in the Front Range of Colorado, geologically known as the Laramide orogeny (Karlstrom et al. 2012). This orogeny lifted the Rockies to their highest elevation of ca. 4,400 m (Mount Elbert), and the periods of glaciation that followed, as well as continual natural erosion from wind and water (Fan et al. 2014), resulted in the vertical terrain known as flatiron formations near Boulder, Colorado located in the southern Rocky Mountains.

The Foothills of the Rocky Mountains throughout the Front Range of Colorado consist of numerous flatiron formations that lack volcanic activity, a unique characteristic that is likely due to a phenomenon known as flat-slab subduction (Karlstrom et al. 2012). This geologic process occurs when a tectonic plate slides under another at a shallow enough angle ($< 30^{o}$) to where the bottom plate continues to glide under the top without breaking through the Earth's mantle. Overall, the lack of volcanic activity, the steep angles of the flatirons, as well as the heterogeneous nature of the sedimentary rocks that form these outcroppings, make the flatirons ideal geologic formations for cliff-dwelling organisms, such as bats.

Bats of the Rocky Mountain Foothills

The Front Range of Colorado is home to nine species of bats (Adams 2010), five of which have been found to roost in the crevices of vertical cliffs (Adams 2003). A few of these cliff-roosting species such as the big brown bat (*Eptesicus fuscus*) and little brown bat (*Myotis lucifugus*) use cliff crevices as maternity roosts, where females nurse and care for their young (Hamilton and Barclay 1994; Adams 2003). Furthermore, Hayes

and Adams (2015) radio-tracked the threatened fringed myotis (*Myotis thysanodes*) to its roosts along the Front Range of Colorado and found that the majority of roosts of this threatened species were located in rock crevices. Not only that, but long-eared myotis (*M. evotis*), small-footed myotis (*M. ciliolabrum*), and Townsend's big-eared bats (*Corynorhinus townsendii*) have also been observed roosting in the Boulder flatirons (Adams and Rolfe 2014).

Previous research has examined how bats select roost sites within caves, and concluded that internal temperature, relative humidity, and distance from the entrance to the cave were important factors in roost selection, and that rocky protrusions were highly correlated with the number of bats roosting in the rocky crevices of the cave (Peñuela-Salgado and Pérez-Torres 2015). These results suggest that the selection of roosting locations within caves by bats involves multiple environmental factors; therefore, it is likely that numerous variables (e.g. temperature, crevices size, anthropogenic disturbance, etc.) will impact where bats choose to roost on the faces of vertical cliffs as well. Many of the species of bat in Boulder form maternity colonies, where numerous females will aggregate together in a single roost, which provides thermoregulatory benefits to the mother and the pup (Solick and Barclay 2006). Because lactation is the most energetically expensive reproductive state of a female (Kurta et al. 1989), finding a roost that will reduce energetic demands by having a more-stable internal temperature is essential for the survival of these bats. Because of the necessity of maternity roosts for many species, anthropogenic disturbances such as entering a roost or walking near a maternity colony can have major consequences on the survival of females and pups, in addition to the abandonment of pups (Adams and Rolfe 2014).

Rock Climbing and Biodiversity of Cliffs

Recreational rock climbing has become increasingly popular since the 1980s, particularly in Boulder Co., Colorado, which has been known for its rock climbing since the turn of the 20th century (City of Boulder, 2014). Today there are numerous types of rock climbing that people do recreationally, which are often specializations of five major types of rock climbing. On the property of the OSMP in Boulder, many individuals practice "traditional" rock-climbing (or "trad" climbing), where the climber uses his/her own climbing holds while ascending the cliffs, which are removed by the climber upon descent. On some of the more-popular cliffs in Boulder there are permanent climbing bolts in place that are used by climbers instead of their own holding gear, in a form of climbing referred to as "recreational" climbing (or "sport climbing"). There are also individuals who practice "free-climbing" in which the climber ascends a steep vertical cliff without any climbing gear at all. In Boulder there are often top-rope climbers as well, who use a two-person system of belaying to ensure the safety of the climbers. In addition to these forms of rock climbing, humans will often practice bouldering, where the climber holds onto (and climbs up) a low-hanging projection of a cliff without any climbing gear (with the exception of a bouldering pad on the ground, if desired). Lastly, many persons (even those who are not "rock climbers") perform scrambling on cliffs, where the individual "walks up" the cliff (often with a significantly shallower grade) using his/her hands and feet and without any climbing gear.

Unfortunately, studies have found evidence that rock climbing causes damage to cliff faces (Krajick 1999), and many investigations have unveiled negative correlations between climbing damage and floral biodiversity in cliff-face ecosystems (Nuzzo 1996;

Kelly and Larson 1997; Camp and Knight 1998; Farris 1998; McMillan and Larson 2002; Müller et al. 2004; Kuntz and Larson 2006; Kuntz and Larson 2005; Bomanowska et al. 2014). Furthermore, work by Holzman (2013) revealed that rock climbing (specifically in the Rocky Mountain Foothills) has led to a decrease in floral biodiversity at the bases of rock-climbing cliffs, which was attributed to the trampling of vegetation along the taluses of cliffs by rock climbers. Furthermore, the chalk used by many rock climbers while ascending cliff faces is left behind, which may change the chemistry and physical properties of the rock that could, hindering the growth of cliff-dwelling flora.

Another group of producers commonly found on vertical cliffs is the lichens. Lichen are composite organisms that are made from a complex symbiosis between a fungus and a species of algae or cyanobacteria, which make the lichen capable of photosynthesis (Barták 2014). Unfortunately, these complex organisms are particularly disturbed by the presence of rock climbers (Baur et al. 2007; Adams and Zaniewski 2012; Studlar et al. 2015), as the physical abrasion often dislodges this fragile flora from its substrate. Anthropogenic destruction of lichen can result in long-term effects on the biodiversity of cliff-faces, as many lichen formations are hundreds to thousands of years old and they help to break down rocky substrates into usable soils. Anthropogenic disturbance via rock climbing was also found to affect populations of cliff-dwelling snails negatively, which was primarily attributed to this activity fragmenting the distribution of the snail's main source of food— oligotrophic lichen (McMillan et al. 2003). Because lichens are fragile and critical resources for herbivores on cliffs, these flora act as great indicators of disturbance on cliff-face ecosystems, especially when examining anthropogenic disturbance due to rock climbing (Kalwu et al. 2005; Zedda et al. 2010).

Many bats are sensitive to ecosystem disturbances, and Medellín et al. (2000) found bat richness and the number of rare bats positively correlated with vegetation cover in neotropical rainforests, with areas of disturbance having a lower biodiversity of bats. Medellín et al. (2000) also concluded that the greater number of phyllostomid bats (a family common to the area of study) was a strong indicator of more-pristine ecosystems. Therefore, it is reasonable to assume that cliffs with a greater richness of roosting bats would indicate areas with less human disturbance.

Some species of bat in the United States are also highly susceptible to anthropogenic disturbance (Lacki 2000). For example, Rafinesque's big-eared bat (*Corynorhinus rafinesquii*) is a rock-roosting species that has been documented to abandon its maternity roost with increased off-trail hiking near its colony (Lacki 2000). Because other members of this same genus, such as Townsend's big-eared bat (*Corynorhinus townsendii*) have been observed roosting in the crevices of the rock-climbing cliffs in Boulder, it is possible that increased anthropogenic disturbance via rock climbing could also cause *C. townsendii* to abandon its maternity roosts. Not only that, but one threatened bat species (*M. thysanodes*) has been observed roosting in vertical cliffs where rock climbing occurs (Adams and Rolfe 2014; Hayes and Adams 2015). According to McCracken (1989), even "innocent disturbances" such as walking past a cluster of hibernating bats or shining a light on a pup, can lead to decreased survival and possible abandonment of offspring. When a disturbance happens near maternity colonies McCracken (1989) claims the following four things may happen: 1) neonates may die by losing their hold and falling to the floor of the roost, 2) the general activity of individuals in the roost increases, which reduces the bat's energy available to reproduction and care

of young, 3) adults will abandon their roost-site (usually when pregnant), where they then select inferior roosts for raising their young, 4) by causing some individuals to leave the colony, the thermoregulatory benefits within the roost decreases (i.e. there are fewer bodies to generate heat), which increases the energetic demand for the individuals that remained in the disturbed roost.

Although rock-climbing is a form of anthropogenic disturbance that occurs on geological features where bats are known to roost and to form maternity colonies, no study has yet investigated the impacts of rock-climbing on the roosting behaviors of bats. Understanding the relationship between rock-climbing and bats in the Foothills of the Rocky Mountains, is essential information needed for the proper management of these at-risk species. This dissertation aims at describing the influence of rock-climbing on the bats of the Rocky Mountain Foothills, in addition to determining the impacts of these bats on the biodiversity of bacteria, fungi, and mesofauna in cliff-face soils, as well as the availability of nitrogen and phosphorus.

Hypotheses

H1 There is an inverse relationship between the frequency of rock-climbing activity and the number (and size) of bat colonies established within cliff-face ecosystems.

H2 The biodiversity of microbiota and mesofauna within the soils of cliff crevices is positively impacted by the presence of roosting bats.

H3 The presence of bats increases levels of otherwise limiting macronutrients in the crevices of vertical cliffs.

CHAPTER II

ROCK CLIMBING AND THE ROOSTING ECOLOGY OF BATS

Abstract

Rock climbing is a relatively novel activity that has brought many people from the cities into nature. However, this new anthropogenic disturbance on cliff faces has been found to impact these rare ecosystems negatively by decreasing the biodiversity of plants, lichens, and invertebrates. Although it has long been known that bats exploit vertical cliffs for roosting, this study was the first to investigate the relationship between rock climbing and bats. During the summers of 2014 – 2017, I compared the impacts of three different levels of rock climbing on the activity of bats in Boulder, Colorado, an area famous for its rock-climbing formations. This area is also important for local populations of bats, including the state-threated fringed myotis (*Myotis thysanodes*), as females often form maternity colonies where they congregate within crevices to care for their young, ultimately creating a more-stable environment (e.g. consistent temperature and humidity) that reduces the energetic demand for rearing their pups.

For this study I used visual observations and acoustic analysis to determine the number of roosts, the quantity of roosting and foraging bats, as well as the species richness and biodiversity of bats at nine vertical cliffs in Boulder, Colorado. I also quantified the percent cover of lichens along and away from climbing routes, which was

used as a measure of anthropogenic disturbance on these cliffs in my multivariate analysis. This variable and other rock-climbing characteristics were correlated with measures of bat activity using Canonical Correspondence Analysis (CCA).

Overall, I located 32 bat roosts among the nine cliffs, with the greatest number of roosts found on sites with moderate-levels of rock-climbing use ($F_{2,105} > 4.98$, $p < 0.009$). With respect to the number of bats roosting in these crevices, the most bats were seen on sites without rock climbing, and with the fewest bats observed on sites with high traffic by rock climbers ($F_{2,105} = 7.25$, $p = 0.0011$). A similar pattern was seen among foraging bats, where more bats were seen foraging around cliffs without evidence of rock climbing, while cliffs with a high degree of rock-climbing traffic had the fewest bats foraging overhead ($F_{2,105} = 8.67$, $p = 0.0003$). Across the four years of this study, I found evidence of six species of bat roosting within the crevices of rock-climbing cliffs, with the little brown bat (*M. lucifugus*) and the western small-footed myotis (*M. ciliolabrum*) being the most commonly observed species in the area. Overall, the species richness of bats was highest on cliffs without rock climbing, followed by sites with moderate-levels of rock climbing, with high-trafficked cliffs having the fewest species of bats ($F_{2,105} = 7.25$, $p = 0.0011$). The Shannon-Wiener Index for bat biodiversity was also higher for sites without rock climbing compared ($F_{2,35} = 12.7$, $p < 0.0001$). With respect to lichens on these vertical cliffs, there was a strong negative correlation between the frequency of rock-climbing on the cliff and the percent cover of lichen ($R^2 \geq 0.72$). The CCA found the percent cover of lichen and seasonal closures to rock climbers had positive relationship with bat activity, whereas climbers per week, trail traffic, and number of climbing routes, were all negatively associated with bat activity.

The results of this study suggest that rock-climbing may have negative impacts on the ecology of cliff-roosting bats, an order of mammals that has been shown to provide important ecosystem services such as pest regulation, pollination, seed dispersal, and nutrient influx. It is critical that wildlife managers monitor bat activity on vertical cliffs where humans are known to rock climb, so proper measures can be taken to regulate the degree of anthropogenic disturbance on cliffs where there is a high degree of bat activity, especially in areas where threatened and endangered species are known to roost.

Introduction

Recreational rock climbing has become an increasingly popular outdoor activity since the 1980s, particularly in Boulder Co., Colorado, which has been known for its climbing since the turn of the 20th century (City of Boulder 2014). This area consists of flatiron formations, which are large rocky outcroppings with a wide base and a steeply sloped face (ca. 45^{o}— Roach 2008). Overall, the steep angles of the flatirons, as well as the heterogeneous nature of the sedimentary rocks that form these outcroppings, make these geologic formations ideal for both rock climbers and cliff-dwelling organisms, alike.

Although they may appear barren from a distance, vertical cliffs are capable of housing high levels of biodiversity and even endemic species. For example, Graham and Knight (2004) identified 163 species of plant both on and near cliffs in Colorado, and 13 species of these plants were found only on the faces of vertical cliffs. The various cracks and crevices of cliff faces serve as different microhabitats within the outcroppings of the rock, which increases habitat heterogeneity, thus providing various places for different

types of wildlife to establish. Therefore, the variability in microhabitats seen on vertical cliffs can have major effects on the diversity of plants, animals, fungi, and bacteria, which can ultimately lead to relatively high levels of biodiversity in a small area of space (Larson et al. 2000).

Unfortunately, studies have found evidence that rock climbing causes damage to cliff faces (Krajick 1999), and many investigations have discovered negative correlations between climbing damage and floral biodiversity in cliff-face ecosystems (Nuzzo 1996; Kelly and Larson 1997; Camp and Knight 1998; Farris 1998; McMillan and Larson 2002; Müller et al. 2004; Kuntz and Larson 2005; Kuntz and Larson 2006; Bomanowska et al. 2014). Furthermore, work by Holzman (2013) revealed that rock climbing (specifically in the Rocky Mountain Foothills) has led to a decrease in floral biodiversity at the bases of rock-climbing cliffs, which was attributed to the trampling of vegetation along the taluses of cliffs by rock climbers. Not only that, but the chalk used by many rock climbers while ascending cliffs may change the chemistry and physical properties of the rock, which may hinder the growth of cliff-dwelling flora.

The Front Range of Colorado is home to nine species of bats (Adams 2010), five of which have been found to roost in the crevices of vertical cliffs (Adams 2003). A few of these cliff-roosting species such as the big brown bat (*Eptesicus fuscus*) and little brown bat (*Myotis lucifugus*) use cliff crevices as maternity roosts, where females nurse and care for their young (Hamilton and Barclay 1994; Adams 2003). Furthermore, Hayes and Adams (2015) radio-tracked the threatened fringed myotis (*Myotis thysanodes*) to its roosts along the Front Range of Colorado and found that the majority of roosts of this threatened species were located in rock crevices. Not only that, but long-eared myotis

(*M. evotis*), small-footed myotis (*M. ciliolabrum*), and Townsend's big-eared bats (*Corynorhinus townsendii*) have also been observed roosting in the Boulder flatirons (Adams and Rolfe 2014).

Many of the species of bat in Boulder form maternity colonies, where numerous females will aggregate together in a single roost, which provides thermoregulatory benefits to the mother and the pup (Solick and Barclay 2006). Because lactation is the most energetically expensive reproductive state of a female (Kurta et al. 1989), finding a roost that will reduce energetic demands by having a more-stable internal environment is essential for the survival of these bats. Because of the necessity of maternity roosts for many species, anthropogenic disturbances such as entering a roost or walking near a maternity colony can have major consequences on the survival of females and pups, in addition to the abandonment of pups (Adams and Rolfe 2014).

Although it has been long known that bats use cliff faces as roosts, this study was the first to examine the impacts of rock climbing (a relatively novel form of anthropogenic disturbance) on the activity of bats on vertical cliffs in Boulder, CO. Specifically, I compared the number of roosts, the quantity of roosting bats, and the species richness and biodiversity of bats among three different levels of rock-climbing use through visual observations and echolocation call analysis; I hypothesized that increased rock climbing would decrease each of these measures of bat activity. I also examined the impacts of rock climbing on the biodiversity and percent cover of lichens, and I hypothesized that increased rock climbing would decrease both lichen biodiversity and cover.

Methods

Sampling occurred at nine vertical cliffs located on Dinosaur Mountain, a protected property of the City of Boulder Open Space and Mountain Parks (OSMP), an area in Boulder, Colorado famous for its flatiron cliff formations and high rock-climbing activity (Fig. 1). I sampled rock-climbing sites that were categorized as either low-use (1 – 100 climbers per year; Veranda, South Ridge, and Bear Creek Spire), medium-use (100 – 500 climbers per year; Red Devil, Lost Porch, and Front Porch), or high-use (>500 climbers per year; Der Zerkle, Dinosaur Rock, and Der Freischutz) by OSMP (Fig. 2). I selected rock-climbing sites based on physical aspects known to attract maternity colonies of bats. Hayes (2011) found the variables aspect and grade to have the largest influence on the establishment of maternity colonies of bats in the Boulder area, with eastern, south, and southwestern aspects of the cliff, and cliffs with steeper grades being those most likely to house maternity colonies of bats. This is likely due to the warmer, more-stable microclimates found within those crevices, which reduces the energy reproductively active females spend on thermoregulation. Therefore, I worked under the assumption that all east-facing cliffs of similar rock type, elevation, and size will have equal probability of housing colonies of bats.

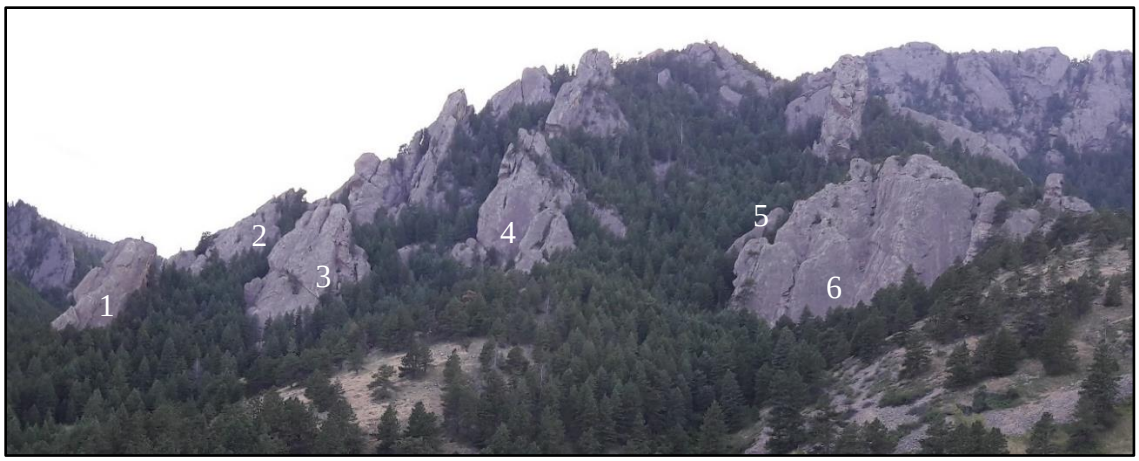

Fig. 1.— Photograph of Dinosaur Mountain, on which the nine sampling cliffs were located; however, only six of the sites are visible in this photograph (1- Bear Creek Spire, 2- Der Freischutz, 3-Der Zerkle, 4- Red Devil, 5- Lost Porch, 6- Front Porch; Photo by A. K. Wilson).

Sampling Sites

All nine sites selected for this study are located on Dinosaur Mountain in Boulder, Colorado, on OSMP property. Dinosaur Mountain is found between Skunk Canyon and Bear Canyon, and the diverse and complex aspects of this mountain reach a summit of 2,243 meters (7,360 feet; Rossiter 1999; Fig. 2). Near the top of Dinosaur Mountain is Mallory Cave, a popular attraction among hikers that has been recently gated and closed to the public to protect a maternity colony of 25 – 30 Townsend's big-eared bats (*Corynorhinus townsendii*) from disturbance. This cave is also closed to prevent transmission of a cold-loving fungus (*Pseudogymnoascus destructans*) that causes White-Nose Syndrome, a deadly tissue disorder that has killed millions of bats across the United States and Canada (Frick et al. 2016). Despite this closure, Dinosaur Mountain is a very popular hiking area that offers numerous rock-climbing routes, some of which are used almost daily.

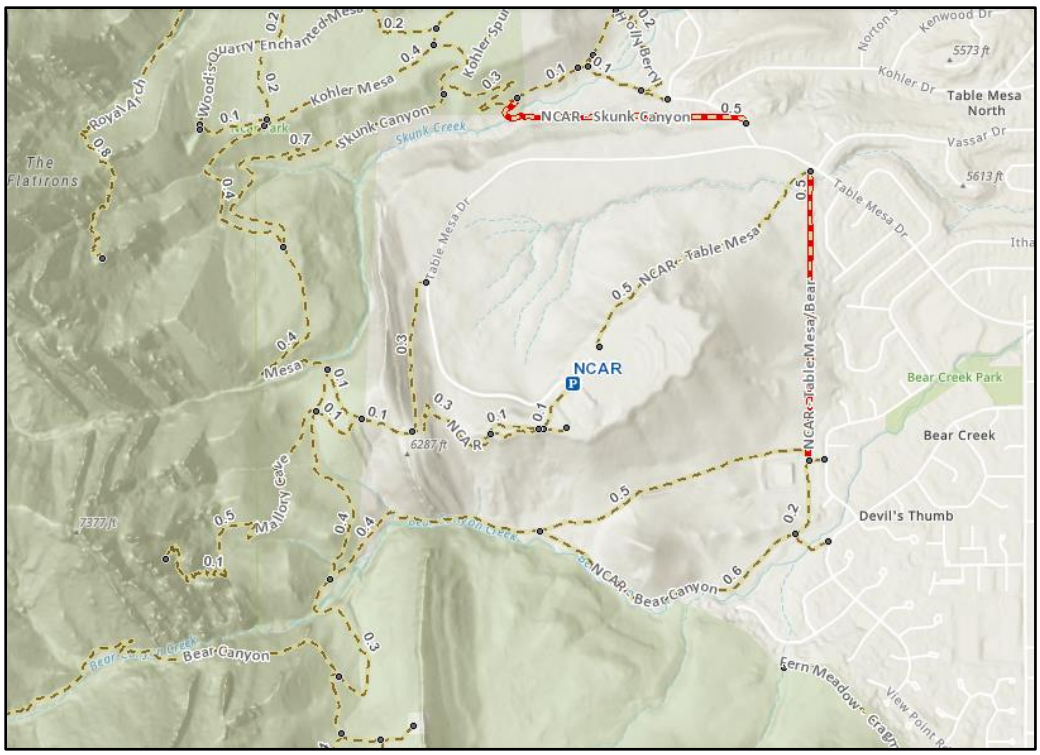

Fig. 2.—Map of OSMP property showing the Mallory Cave trail that runs up Dinosaur Mountain. Image from https://maps.bouldercolorado.gov/osmp-trails/.

Bear Creek Spire. A rock-climbing site that is categorized as low-use by OSMP and that often has seasonal closures due to raptor nesting (Table 1). This cliff is not located directly on a main (Mallory Cave) trail like most sites; but rather, hikers and climbers must veer-off the Bear Canyon Trail (toward the northeast) to reach this climbing rock. This rock is the southeast-most site among those sampled, and it is found at the edge of the forest on Dinosaur Mountain and the riparian habitat along Bear Creek. The vegetation at the base of Bear Creek Spire includes (but is not limited to) riverbank grape (*Vitis riparia*), poison ivy (*Toxicodendron radicans*), blackberry shrubs (*Rubus* sp.), smooth sumac (*Rhus glabra*), ponderosa pine (*Pinus ponderosa*), and apple species (*Malus* sp.). Bear Creek Spire is a steep cliff (practically vertical) that provides numerous surfaces for traditional rock-climbing, as well as one slope (found along eastern rim of the cliff) that is an intense scramble partially up the height of the rock.

South Ridge. This is the southwestern-most site sampled, which is also categorized as low-use by the OSMP (Table 1). This rock-climbing location is found directly off the Bear Creek Trail but is only reached after crossing Bear Creek and walking another mile along the Bear Canyon Trail until the path connects with Bear Creek once again. To reach the climbing areas at South Ridge, one must scramble across riparian boulders, pass through a grove of *T. radicans*, then ascend a steep, long, and loose talus slope. This unpleasant/unsafe trek and relatively short cliffs at South Ridge likely contribute to this site being categorized as low-use by rock climbers. The climbing rocks of South Ridge stretch from north to south and are found between the edge of the forest on Dinosaur Mountain and the riparian area along Bear Creek. Species of plant found at this location include (but are not limited to) *P. ponderosa*, *V. riparia*, *R. glabra*, and aspens (*Populus* sp.). Once one has reached the climbable cliffs at South Ridge, many of the areas along these ridges are an easy-to-moderate scramble to the summit.

Veranda. Another site categorized as low-use by the OSMP is Veranda, which is the first major rock found along the popular Mallory Cave Trail on Dinosaur Mountain (Table 1). Veranda is toward the interior of the forest on Dinosaur Mountain, but this rock is flanked by grasses (Poaceae) on three sides. However, the area also contains *P. ponderosa*, common Juniper (*Juniperus communis*), and catnip (*Nepeta cataria*), with the northern edge of Veranda touching the edge of the interior forest on Dinosaur Mountain. Once one walks through the grass field around Veranda, it is a simple scramble to the top of this cliff; however, there does not appear to be any tall and/or steep enough areas on this cliff for either traditional or recreational rock climbing.

Front Porch. Two of the three medium-use cliffs sampled for this study were located along a less-taken trail called Porch Alley (Table 1). The start of Porch Alley can be found along Mallory Cave Trail ca. 0.25 miles after its junction with Mesa Trail. This indiscrete trail extends through the Ponderosa pine forest and is lined with blackberry bushes (*Rubus* sp.), Oregon holly grape (*Mahonia repens*), and golden currant (*Ribes aureum*). Front Porch is the first cliff found along Porch Alley and it has the largest eastern face of any of the nine cliffs sampled. There are a few places along the eastern face of the cliff where it is a moderate scramble partially up the cliff (usually ca. 1/3 of the way to the summit), and toward the northern point of the eastern face of Front Porch one can easily scramble to the top.

Lost Porch. Continuing along the Porch Alley trail (which extends behind the southern and western sides of Front Porch) the next cliff found is Lost Porch, another site categorized as medium-use by the OSMP (Table 1). Lost Porch has a flattened area halfway up the western side of the cliff that allows for scrambling partially up the rock to view the majority of the eastern slope from above. Unlike most of the other cliffs sampled, the eastern face of Lost Porch has a slope that is too steep for scrambling, but too shallow and smooth for traditional rock climbing. Front Porch is the most isolated cliff sampled during this study, and it is the most interior site within the ponderosa pine forest on Dinosaur Mountain.

Red Devil. Another medium-use cliff is Red Devil, which is the steepest and tallest cliff sampled during this study (Table 1). Although the top (western face) of Red Devil is easily accessible from the top of the Mallory Cave Trail, the large, steep eastern face is only reachable via scrambling around the top of the cliff, or by hiking through un-

blazed forests to get to the eastern base of this rock. Near the top of the western side of Red Devil, the habitat is primarily talus slopes with medium- to large-sized boulders, with the occasional *P. ponderosa*, *N. cataria, R. aureum*, and other small herbaceous plants.

Der Zerkle. All three of the cliffs categorized as high-use by the OSMP that I sampled are found along the main Mallory Cave Trail (Table 1). Der Zerkle, is a high-use site that is the next cliff after Veranda along the Mallory Cave Trail. This eastern face of Der Zerkle is closed during the summer due to an established colony of fringed myotis (*M. thysanodes*), a state-threatened species. This cliff has the characteristic flatiron shape, with a large crux running horizontally ca. 2/3 of the way up the rock. Although the eastern face of this cliff is closed during the summer, the western face is among the most commonly climbed areas on Dinosaur Mountain, and it even has permanently bolted holds for recreational rock climbers.

Dinosaur Rock. This is a cliff categorized as high-use by OSMP (Table 1) that often has seasonal closures due to nesting raptors (with the exception of 2017 when the birds moved locations). Dinosaur Rock is a fairly-tall and steep cliff that is near the top of Dinosaur Mountain and at the edge of its ponderosa-pine forest. Between Dinosaur Rock and Bear Creek Spire (which is located directly south) lie multiple short ridges that meet the steep slopes of talus of Bear Creek Spire below. Although the western face of Dinosaur Rock is within the forest, the eastern face is open to Bear Canyon. This site has *P. ponderosa* and *J. communis* growing throughout.

Der Freischutz. This cliff is located just after Dinosaur Rock along the Mallory Cave Trail and is also categorized as high-use by OSMP (Table 1). This cliff runs

primarily north to south, extending from the edge of the forest toward Bear Canyon. Der Freischutz has a relatively large eastern face that is accessible directly off the primary trail, and this site has numerous boulders along the base of cliff, which creates large talus-slope crevices that are not found at other locations. Although usually open for rock climbing during the summer months, Der Freischutz was closed during the summer of 2017 due to raptor nesting. Vegetation in the area includes *P. ponderosa*, *J. communis*, *N. cataria*, and some grasses (Poaceae).

Table 1.—Location information of the nine vertical cliffs sampled in this study.

Site	OSMP Use-Level	Trail	Landscape	Dominant Vegetation
Bear Creek Spire	Low	Bear Canyon	Forest Edge/Riparian	*Vitis riparia*, *Toxicodendron radicans*, *Rubus* sp., *Rhus glabra*, *Malus* sp., *Pinus ponderosa*
South Ridge	Low	Bear Canyon	Forest Edge/Riparian	*V. riparia*, *R. glabra*, *Populus* spp., *P. ponderosa*
Veranda	Low	Mallory Cave	Trail Edge/Forest Interior	*P. ponderosa*, *Juniperus communis*, *Nepeta cataria*, numerous Poaceae
Front Porch	Medium	Porch Alley	Forest Interior	*P. ponderosa*, *Rubus* sp., *Mahonia repens*, *Ribes aureum*
Lost Porch	Medium	Porch Alley	Forest Interior	*P. ponderosa*, *Rubus* sp., *Mahonia repens*, *Ribes aureum*
Red Devil	Medium	Mallory Cave	Trail Edge/Forest Interior	*P. ponderosa*, *N. cataria*, *R. aureum*
Der Freischutz	High	Mallory Cave	Forest Edge	*P. ponderosa*, *J. communis*, *N. cataria*, some Poaceae
Der Zerkle	High	Mallory Cave	Trail Edge/Forest Interior	*P. ponderosa*, *J. communis*, some Poaceae
Dinosaur Rock	High	Mallory Cave	Forest Edge	*P. ponderosa*, *J. communis*

Fig. 3.—Nine sites sampled on Dinosaur Rock with OSMP rock-climbing use-levels: blue = low-use (1 – 100 visits per year), yellow = medium-use (100 – 500 visits per year), and red = high-use (>500 visits per year). Image taken from Google Earth.

Quantifying Anthropogenic Disturbance on Cliffs

In order to determine whether the categories of rock-climbing use (low-use, medium-use, and high-use) as designated by OSMP are in accordance with the amount of rock climbing occurring on these cliffs during the summer months of 2017, I assessed the frequency of climbing on my nine cliff sites using a Brinno® time-lapse camera, a device that is typically used to monitor the progress of construction sites. This small, weather-resistant camera has an angle of view up to 140^{o}, a digital screen for focusing the panoramic area of interest, settings for taking photos every 1 sec. to 24 hrs. and a two-month battery life. I used three of these cameras to photograph the cliff face of one 'low-use,' one 'medium-use,' and one 'high-use' site simultaneously, by affixing a Brinno® time-lapse camera to a tree facing the eastern face of each rock-climbing cliff. These cameras were placed in areas that maximized the surface area of the cliff visible to the camera, and these cameras were set to take a photo of the cliff every minute (between sunrise and sunset) for a period of one week, after which these cameras were relocated to another set of three cliffs (one low-, medium-, and high-use rock, respectively) to be monitored in the same fashion for one week. This pattern of sampling was repeated for nine weeks, allowing each site to be sampled for a total of three weeks, from June – August 2017.

The site-pairing for photographing (i.e. which low-, medium-, and high-use site will be sampled simultaneously) was determined by a randomization function in Microsoft Excel®; however, to keep the time-period between sampling bouts consistent for each rock, each randomized group of cliffs remained clustered together for the entirety of the study. After each rock was photographed for one week, I downloaded the

corresponding photos (in a compressed time-lapse video) to determine the frequency of rock climbing (number of rock climbers/week) at each location. In addition, I used these photos to determine where the primary rock-climbing routes were on these cliffs, which provided insight on the distribution of bat roosts relative to these routes.

Assessing the Activity of Bats on Cliff Faces

For four summers (2014 – 2017), nine rock-climbing cliffs in OSMP were sampled for three consecutive nights (weather permitting) to assess the level of bat activity (*i.e*. total of 12 nights of observation per site). Two observers sat within 5 meters from the base of each cliff and at least 15 meters apart from one another, to maximize the surface area observed of the eastern face of the cliff, while minimizing visual overlap between observers. Beginning at sunset, each observer scanned the cliff face for emerging bats to determine the approximate location of bat roosts on the cliff. These roost locations were recorded onto an image of the eastern cliff face, which then were manually translocated into a Google Earth file of the cliffs at a later time. While scanning the cliff faces, the two observers recorded the echolocation calls of the emerging bats. This was done by mounting a SM2Bat+ detector (Wildlife Acoustics, Maynard, Massachusetts) within 1 m from where the observer sat, using bungee cords, rocks, logs, etc., prior to the observation period each night (recording settings: gain = 0 dB, dig HPH = fs/24, dig LPF = off, Trg Lvl = 18SNR, and Trg Win = 2.0s). Both SM2Bat+ detectors had SMX-UT microphones (affixed with an acoustic horn for unidirectional recording) positioned at an angle approximately equal to that of the cliff face (visually estimated by the observer; Fig. 3). When possible, recorded calls of the

emerging bats observed were identified, which were then used to determine the species of bat roosting in the cliffs (Fenton and Bell 1981).

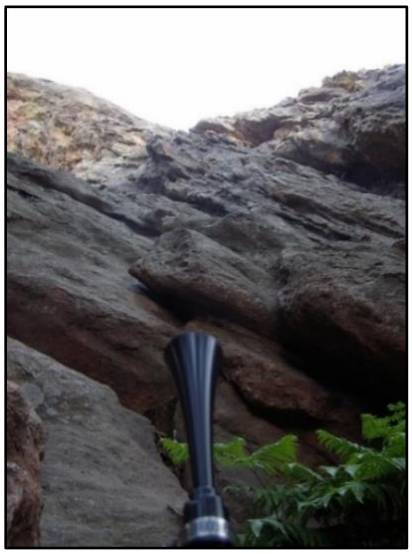

Fig. 4.—Example of the positioning of the unidirectional horn affixed to the SM2BAT+ bat detectors, which were used to record the echolocation calls of emerging bats (Photo by A. K. Wilson).

Visual observations and recording bouts continued for 45 minutes after published sunset times for Boulder, CO, to maximize the chances of recording emerging bats while limiting the number of calls potentially recorded from foraging individuals that were not roosting on the survey rock. However, bats seen foraging in the area (i.e. flying in an acrobatic way and/or circling in the same area for an extended period, >30 sec.) were also recorded and statistically compared. To identify which species of bat emerged from the respective rock face, sonar calls were analyzed via SonoBat 3.1 (U.S. West, Arcata, California), using 76 different parameters for each call (with a discriminant probability threshold set to 0.9, and the acceptable call quality set to 0.8). Calls were analyzed after being run through the SonoBat Batch Attributer and Scrubber to remove noise files. The sonograms of automatically identified calls were visually vetted (by comparing calls to the Sonobat Western Reference Views), to ensure that the species-level identification of

the call aligned with the specifications of the identified species (Fig. 6). For calls not automatically identified, I attempted to classify these files manually by assessing the sonograms and the high frequency, low frequency, bandwidth (high freq. – low freq.), frequency at knee, high freq. to knee slope, and knee to low freq. of the calls (Fig. 5). These calls were used to determine the species richness of bats, as well as the biodiversity of bats at these locations using the Shannon-Wiener Index (H), where p_i is the proportion of bat species *i* in the assemblage.

$$H = -\sum p_i (\ln p_i)$$

Because the larger climbing rocks have more surface area on which bats may roost, visual observations on the number of emerging bats were standardized across survey sites by dividing 1) the number of roosts, 2) the quantity of roosting bats and foraging bats, and 3) the richness and biodiversity of bat species by the approximate surface area of the observed area of the cliff. The distribution of bat roosts on Dinosaur Mountain was also mapped via Google Earth, which will likely be used by OSMP staff for management protection of these colonies in the future.

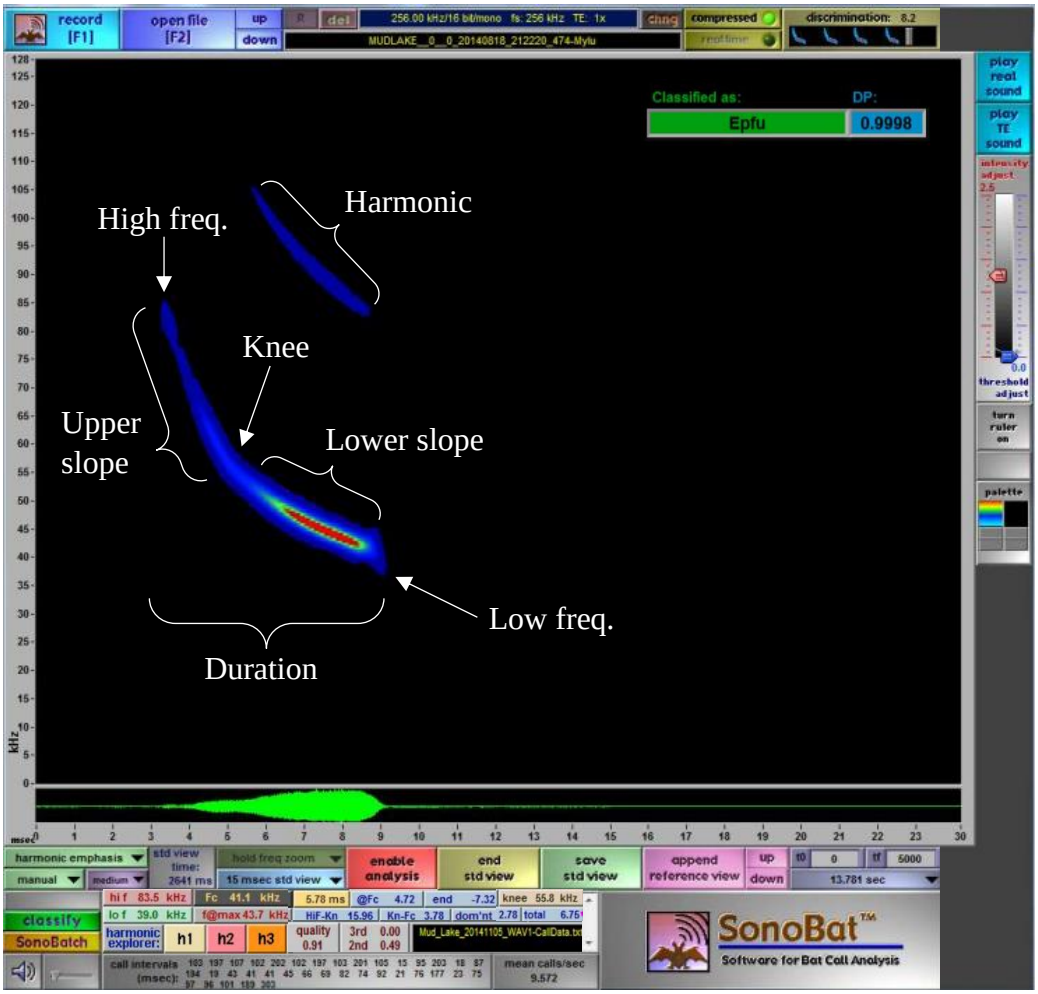

Fig. 5.—Sonobat 3.1 software showing the different attributes measured during call analysis to determine the species of bat emerging from the cliffs. High freq. = the highest frequency of the primary call; knee = where there is an obvious change in angle of the call; low freq. = the lowest frequency in the primary call; duration = time (msec) for the call to complete (Image created by A. K. Wilson).

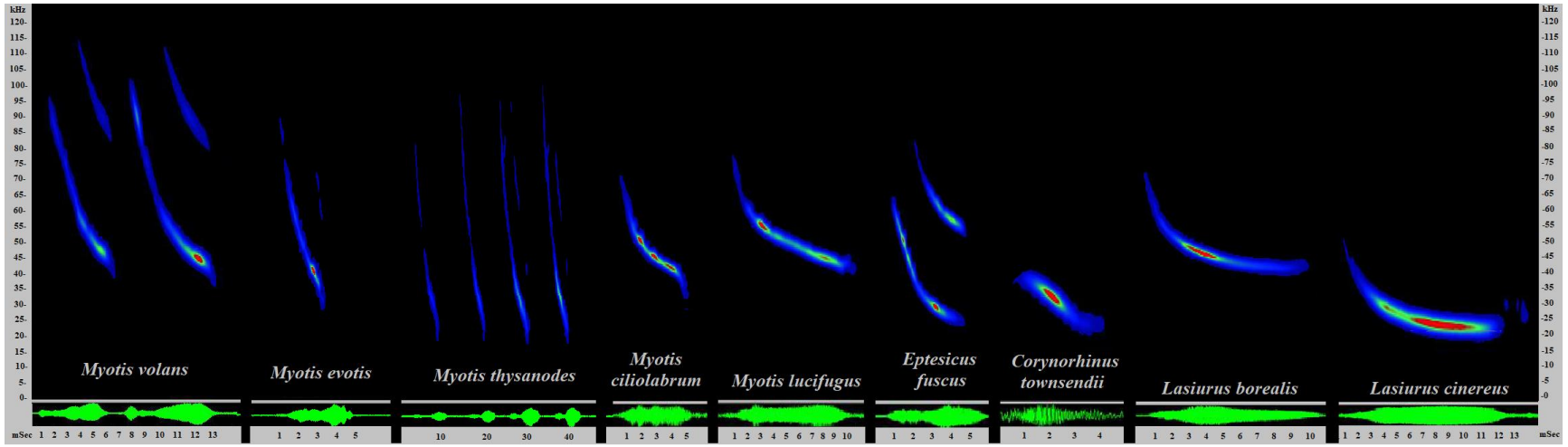

Fig. 6.—Echolocation call profiles for the nine species of bat found on Dinosaur Mountain including the approximate frequency of calls (kHz) as well as the call duration (msec). These criteria were used to confirm the species-level identification of the calls recorded and analyzed in SonoBat 3.1 (Image created by A. K. Wilson).

Rock Climbing and Lichen Biodiversity

Because cliff-dwelling lichen are easily damaged and dislodged by rock climbers, I also examined the anthropogenic disturbance to the surface of these vertical cliffs (via rock climbing) by comparing the biodiversity (determined by the richness and relative surface area) of lichen assemblages along and away from climbing routes. The biodiversity of lithophilic lichen was then used as an independent measure of disturbance on that rock for the multivariate analysis (CCA).

The photos acquired from the time-lapse cameras were then used to determine areas for sampling lichen along climbing routes, as well as for identifying areas of the rock that were unclimbed. Once the commonly used climbing routes were identified on these vertical cliffs, I superimposed a 0.5-m x 0.5-m grid over an image of the cliff labelling accessible quadrats on the cliff's grid with either a letter (along climbing routes and +/- two meters from the route) or a number (for the unclimbed areas of the cliff). I then used a randomization function in Microsoft Excel® to determine which five alphabetical squares and five numerical squares were to be surveyed at each cliff sites. Only quadrats that were no higher than 10 m above the ground were labelled and put forth for randomized sampling. Each selected quadrat was photographed in the field at distance from the surface of the cliff so that the entire quadrat was in the field of view of the camera (ca. 1 m away from cliff). From these photographs I estimated the richness of lichens based on the color and texture of the lithophilic organisms, and I estimated the relative surface area of each species of lichen by estimating the proportion of the photograph's total area that was taken-up by each single species (i.e. lichens of the same

color and texture were classified as one species). I used a Mann-Whitney U-test (α = 0.05) to compare the species richness of lichens between climbed and unclimbed areas.

The surface area of each type of lichen in every quadrat was used as the relative abundance of each species in the assemblage (p_i), which I used to estimate the biodiversity of the lichen via the Shannon-Wiener Index (H):

$$H = -\sum p_i (\ln p_i)$$

I then used a *t*-test (α = 0.05) to compare the biodiversity of the lichen assemblages between rock-climbing routes and undisturbed areas. Lastly, the percent lichen cover was used as a measure of anthropogenic disturbance for my multivariate analysis, which was used to examine the impacts of rock-climbing characteristics on the roosting behaviors of bats on Dinosaur Mountain.

Multivariate Analysis

To test if the number of roosts, the quantity of emerging individuals, and the richness of bat species observed at these nine rock-climbing sites were correlated with climbing characteristics (e.g. OSMP use-level, number of climbing routes, average route difficulty, percentage of traditional routes, seasonal closures, frequency of rock climbing, and percent cover of lichen on the rock), I used Canonical Correspondence Analysis (CCA— Kuntz and Larson 2005; Kuntz and Larson 2006; Adams and Zaniewski 2012; Kolb et al. 2015; Peñuela-Salgado and Pérez-Torres 2015). CCA is a multivariate technique that uses a non-linear algorithm to ordinate measures and to correlate the independent variables with multiple dependent variables. Although both CCA and principle components analysis (PCA) are used to reduce the number of explanatory variables in a data set, only CCA incorporates regression analyses that compare the

relationships between two (or more) variables. This allowed for the separation of the independent from the dependent variables and thereby tested for correlations specifically between these two groups of values. Furthermore, CCA incorporates an algorithm (whereas PCA has an assumption of linearity) that better reflects the non-linear relationships typically observed in ecological systems. The major limitation to CCA, however, is that it is not based on hypothesis testing, and instead identifies the strongest relationships between two datasets (i.e. independent vs. dependent variables).

Results

Bat activity was observed on Dinosaur Mountain for four consecutive summers (2014 – 2017), totaling 12 nights of observation for each of the nine sites, and 108 nights of observation total. The nine cliffs were all located on Dinosaur Mountain and were originally selected due to their use-level by rock climbers as categorized by OSMP (Fig. 3). Each of these nine sites had bats roosting in the crevices of the eastern face, with the location of these roosting sites remaining relatively stable over the four-year period for most sites. Because the nine cliffs used in this study were of variable size (Table 2), the number of roosts, the quantity of emerging bats, and the richness and biodiversity of bats at each site were divided by the surface area observed at each cliff to standardize all measurements to 1-m^2 for statistical comparisons.

Table 2.—The surface approximate area observed at each of the rock-climbing sites from 2014 to 2017.

OSMP Use Level	Rock-Climbing Site	Surface Area Observed
Low	Bear Creek Spire	881.59 m^2
	South Ridge	3,764.02 m^2
	Veranda	1,273.66 m^2
Medium	Front Porch	3,852.55 m^2
	Lost Porch	807.8 m^2
	Red Devil	2,131.8 m^2
High	Der Freischutz	2,579.19 m^2
	Der Zerkle	1,332.88 m^2
	Dinosaur Rock	1,490.26 m^2

Quantifying Anthropogenic Disturbance on Cliffs

Time-lapse cameras were placed at each of the nine cliff sites for three weeks during the summer of 2017, but evidence of rock climbing was not found at all sites (Table 3). The site that had the greatest disturbance frequency was Der Zerkle; however, most of the climbing took place on the western aspect of the rock due to a seasonal closure on its eastern surface during the summer months when this quantification occurred. Dinosaur Rock had the greatest human activity on the eastern face of any cliff (Fig. 7; Table 3), and the western side of this rock is also frequently climbed (personal observation; but this aspect was not photographed in this study). Veranda had the second-greatest number of climbers on its eastern face, which was interesting because this site is currently categorized at low-use by the OSMP (even though a person can easily scramble up the slope; Fig. 8; Table 3). Conversely, the sites Front Porch (Fig. 9; Table 3) and Lost Porch were classified as medium-use by OSMP, however, only two persons were found scrambling up Front Porch over the three-week filming period, and

nobody was seen climbing Lost Porch at all. Similarly, Der Freischutz (Fig. 10) has been classified as high-use by OSMP, but I recorded only one person climbing this rock (Table 3). Numerous people were climbing on the western face of Der Zerkle, and a couple people were returning from the closed eastern face of the cliff (Fig. 11; Table 3). Not surprisingly, no climbers were seen on Bear Creek Spire nor South Ridge, which is likely due to the closure of Bear Canyon Trail during summer 2017, the path that must be taken to get to either of these locations.

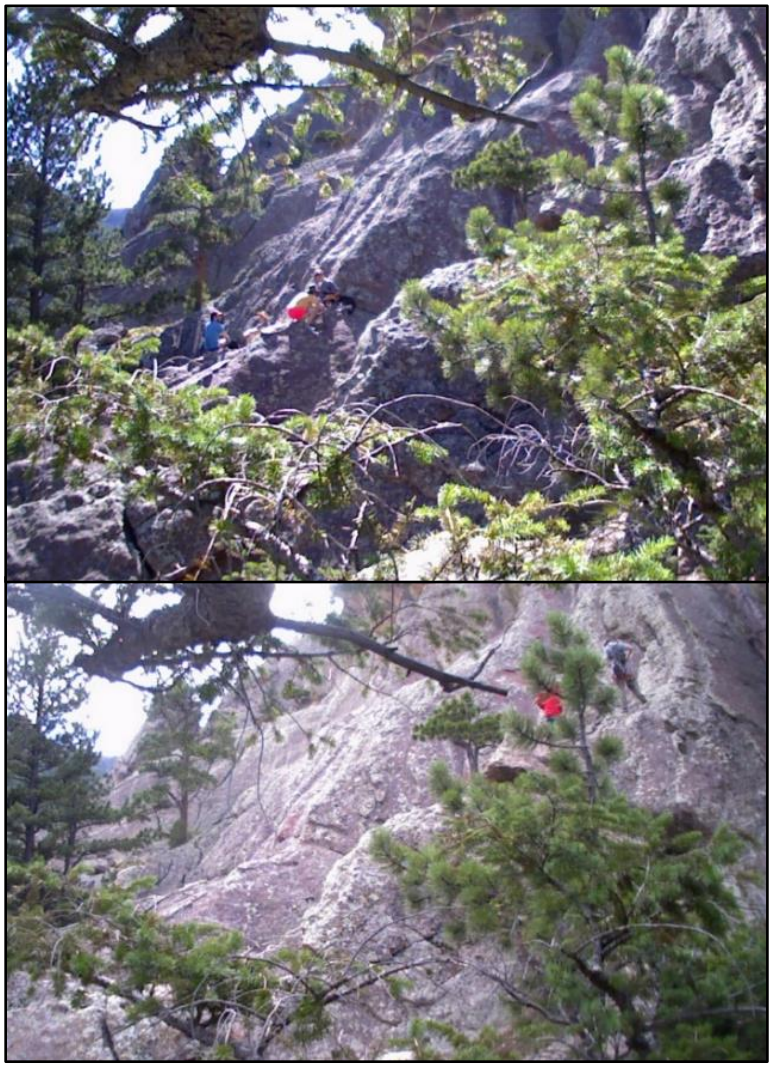

Fig. 7.—Evidence of people scrambling on the south-eastern surface of Dinosaur Rock (a high-use site), which was captured using the time-lapse cameras.

Fig. 8.—Photo of a person scrambling on the eastern surface of Veranda (a low-use site), which was captured using the time-lapse cameras.

Fig. 9.—Photo of a person climbing the eastern surface of Front Porch (a medium-use site), which was captured using the time-lapse cameras.

Fig. 10.—Photo of a person climbing the eastern surface of Der Freischutz (a high-use site), which was captured using the time-lapse cameras.

Fig. 11.—Photographs of people climbing on the western face (top) of Der Zerkle (high-use site), as well as evidence of people returning from the closed eastern face of the cliff (bottom), which was captured using the time-lapse cameras

By multiplying the average number of climbers per week (as captured by the time-lapse photography) on Dinosaur Mountain by the number of weeks in a year (52), my data corroborate with the majority of the classification of rock-climbing use by OSMP (Table 3). For example, my findings suggest that both Bear Creek Spire and South Ridge are infrequently used by rock climbers, as previously categorized by OSMP,

and both Der Zerkle and Dinosaur Rock were classified as high use by OSMP, which was supported by my photography. Some discrepancies did exist, however. Lost Porch is classified as medium-use by OSMP, but according to my data, this cliff was likely a low-use site in 2017, as I found no evidence of any climbing on this rock. Similarly, Front Porch was also categorized as medium-use by OSMP but because of the relatively few people photographed climbing on this cliff, it may be more-appropriately classified as low-use by climbers. Der Freischutz surprisingly had relatively few people climbing on it, suggesting it may be better classified as a low-use site rather than high-use; however, during the year climbing photos were taken, Der Freischutz had a seasonal closure (although *not* when the photos were taken). Finally, Veranda is a cliff that was categorized as low-use by OSMP, presumably due to the limited height and shallow angle of this rock. However, (possibly due to the easy scramble at this location) Veranda had the third highest frequency of climbers, suggesting that although this cliff is not "climbed" often, its level of anthropogenic disturbance may be better classified as high-use.

Table 3.—Results of the rock-climbing data obtained from the Brinno® time-lapse cameras among the nine sites sampled, organized from least climbed to most climbed.

Climbing Site	Frequency of Rock Climbing (Climbers/Week)	OSMP Use-Level	Photo-Based Use-Level
Der Zerkle	0[a]	High	Zero
Bear Creek Spire	0	Low	Zero
Lost Porch	0	Medium	Zero
South Ridge	0	Low	Zero
Der Freischutz	0.33	High	Low
Front Porch	0.66	Medium	Low
Red Devil	1	Medium	Low
Veranda	4.5	Low	High
Dinosaur Rock	17	High	High

[a-] Western face had high activity of rock climbing at 75 climbers per week.

In addition to photographing humans near these cliff sites, the Brinno® time-lapse cameras also photographed two sweat bees (Diptera; Syrphidae), one at Der Freischutz and one near Bear Creek Spire (Fig. 12). These cameras also caught a black bear (*Ursus americanus*) using the trail between Bear Creek Spire and South Ridge, and a mountain lion (*Puma concolor*) at the base of Front Porch (just 57 mins. prior to a human being photographed in the same location; Fig. 12).

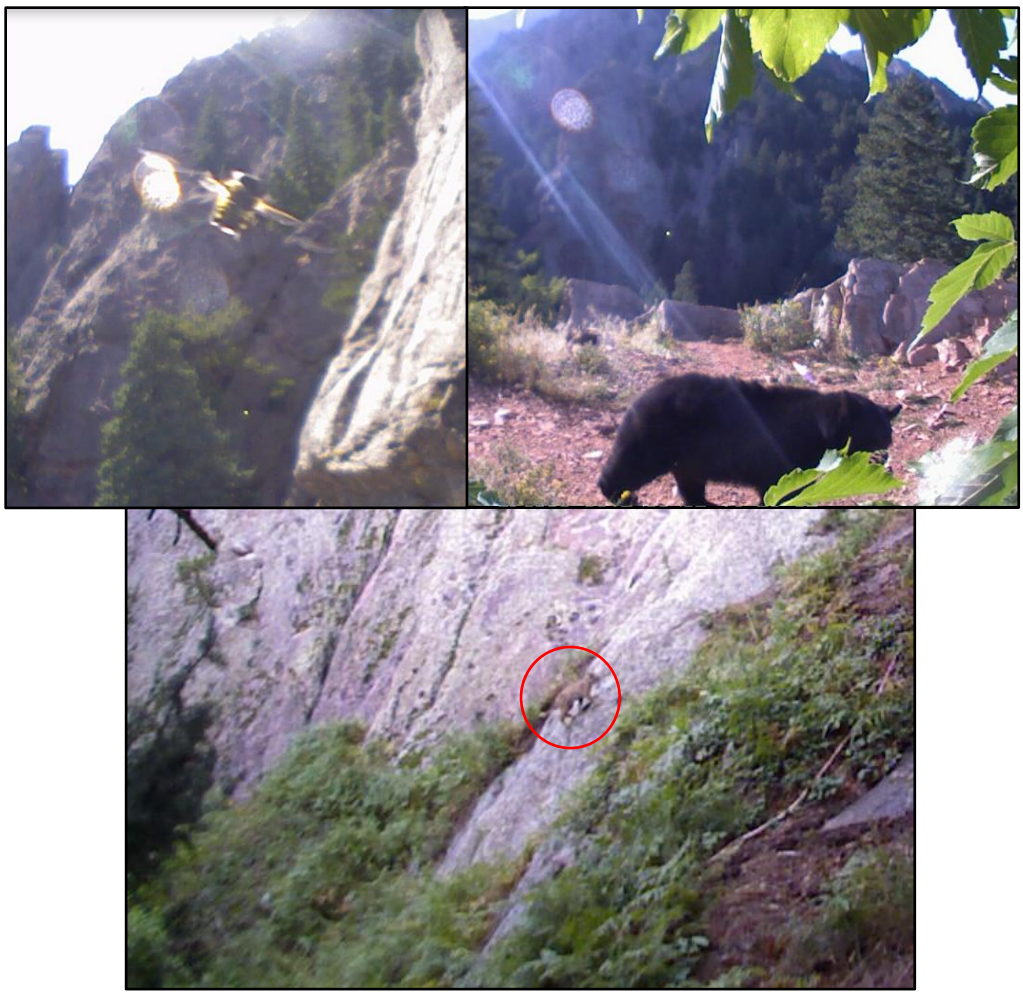

Fig. 12.—Animals captured using time-lapse cameras, a syrphid fly, a black bear (*U. americanus*), and a mountain lion (*P. concolor*).

Species of Bat Roosting on Dinosaur Mountain

Over the 108 nights of observation, I witnessed six species of bat using the cliffs as roosts: the big brown bat (*Eptesicus fuscus*), western small-footed myotis (*Myotis ciliolabrum*), western long-eared myotis (*M. evotis*), little brown bat (*M. lucifugus*), fringed myotis (*M. thysanodes*), and long-legged myotis (*M. volans*;

Fig. **13**). Tree-roosting species such as the hoary bat (*Lasiurus cinereus*), eastern red bat (*L. borealis*), and silver-haired bat (*Lasionycteris noctivagans*) were also recorded

occasionally at these sites, but they were not included in the analysis because they were not using rock-climbing sites as roosts.

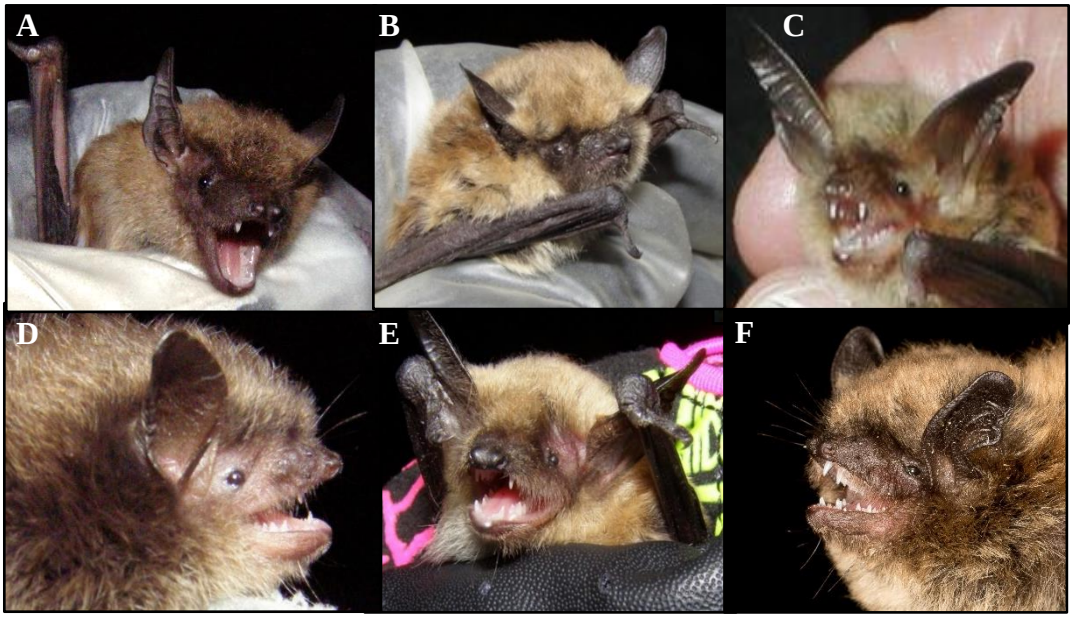

Fig. 13.—Portraits of the six species of bat found roosting on the nine cliffs sampled in this study: A) big brown bat (Eptesicus fuscus), B) western small-footed bat (Myotis ciliolabrum), C) western long-eared myotis (M. evotis), D) little brown bat (M. lucifugus), E) fringed myotis (M. thysanodes), and F) long-legged myotis (M. volans). Photos A–E were taken by A. K. Wilson; photo F was taken by Jon Hall and acquired from Mammalwatching.com.

Townsend's big-eared bat (*Corynorhinus townsendii*) is also found in the area, and 25 – 35 individuals are known to roost inside Mallory Cave. Outside of this cave, however, only a single *C. townsendii* was observed roosting in an open cave-like structure of Der Freischutz during the first three years of this study. Unfortunately, this individual was absent from the location when I sampled Der Freischutz in 2017 and was replaced by novel graffiti on the walls of the cave (Fig. 14). One piece of graffiti alluded to the act of smoking marijuana inside the rocky outcropping, which could explain why this bat was no longer using this location in 2017.

Fig. 14.—Solitary *C. townsendii* found roosting in Der Freischutz the first three years of this study. In the fourth year, the bat was not present when the cave was checked (on 11 August 2017), but graffiti was found in the small cave indicating significant disturbance.

Bat Richness and Biodiversity at the Nine Cliff Sites

Of the 4,258 echolocation calls I recorded from bats from 2014 – 2017, ca. 42% of those calls could not be identified to the level of species. The identified echolocation calls were primarily *M. lucifugus* (40.1%) and *M. ciliolabrum* (31.4%), with *M. evotis* (5.4%) and *M. volans* (2.0%) being the most-rarely recorded species (Fig. 15). At my survey sites, species richness ranged from 1 – 6 species (Table 4). Regardless of the species, all bats left their roosts for the night within 40 mins after sunset. The peak emergence time was ca. 20 – 30 minutes after sunset for most sites, with the exception of Der Zerkle, Dinosaur Rock, and Front Porch, which all had peak emergence times ca. 30 – 40 minutes after sunset. The first species to emerge from the cliffs after sunset was

most often *M. ciliolabrum*, followed by *M. lucifugus*, and then *E. fuscus*, while *M. thysanodes* was often the last to leave the cliff, if present at that site. Although *E. fuscus* is often the first species to emerge for the night, this was not always the case in this study. This was likely because *E. fuscus* comprised just ca. 10% of the calls, which were often recorded after some of the early-emerging *M. lucifugus* and *M. ciliolabrum* individuals. If more E. fuscus roosted in these cliffs, I would expect the majority of the individuals to emerge before most of the *Myotis* individuals in the area.

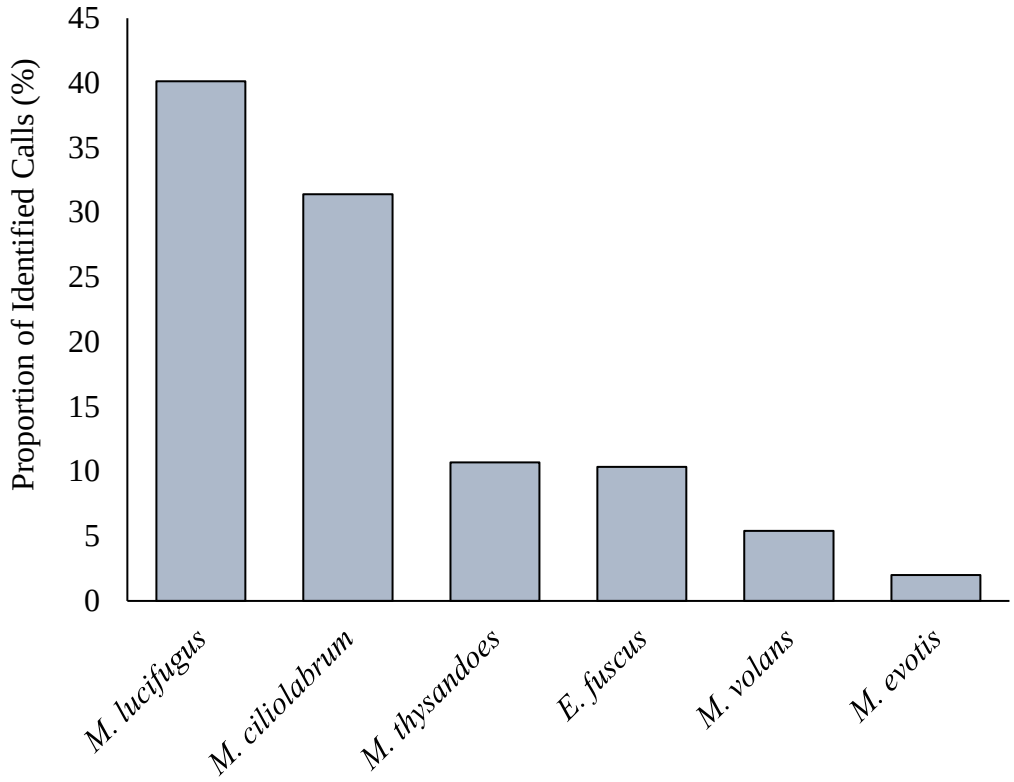

Fig. 15.—Proportion of echolocation calls identified for each species of bat.

Table 4.—The species richness of bats recorded at each of the nine sites between 2014 and 2017.

Use-level	Climbing Site	2014	2015	2016	2017
Low	Bear Creek Spire	3[a]	4[a]	5[a]	4
	South Ridge	1	3	1	1
	Veranda	2	2	2	2
Medium	Front Porch	3	3	4	4
	Lost Porch	2	3	4	4
	Red Devil	3	3	3	3
High	Der Freischutz	3	3	3	4[a]
	Der Zerkle	2[a]	5[a]	6[a]	6[a]
	Dinosaur Rock	2[a]	4[a]	5[a]	3

[a-] Years when site had a seasonal closure for rock climbing.

If all six species of cliff-roosting bats were found at any site all four survey years, the total richness value would equal 24. However, there was no site in which this was the case (Tables 4 and 5). Total site richness across the four years ranged from 6 at South Ridge (or 1.5 species per year) to 19 at Der Zerkle (or 4.75 species per year), with the average of 12.8 across all sites, or approximately three species per site per year. In addition to South Ridge (total richness of 6), Veranda also had a relatively low total richness of 8. Both sites have relatively short cliffs with shallow angles, but the degree of anthropogenic disturbance via climbing differed between these two areas drastically.

Table 5.—The presence of cliff-roosting species at the nine rock-climbing sites sampled. Black X's denote observations of a species in 2014, red X's correspond with observations in 2015, blue X's represent observations in 2016, green X's marks observations in 2017.

OSMP Use-level	Climbing Site (OSMP use rating)	*Eptesicus fuscus*	*Myotis ciliolabrum*	*Myotis evotis*	*Myotis lucifugus*	*Myotis thysanodes*	*Myotis volans*	Total Richness
Low	Bear Creek Spire (1)	X	XXXX	X	XXXX	XXX	XXX	16
	South Ridge (1)	X	X		XXXX			6
	Veranda (0)	X	XXX		XXXX			8
Medium	Front Porch (1.5)	XXXX	XXX		XXXX		X XX	14
	Lost Porch (1.3)	XX	XXX	X	XXXX		XXX	13
	Red Devil (1.8)	XX	XX X		XXXX		XXX	12
High	Der Freischutz (2.3)	X X	X	X	XXXX	X	XXXX	13
	Der Zerkle (3)	XXX	XX	XXX	XXXX	XXXX	XXX	19
	Dinosaur Rock (2.6)	XXXX	XX		XXXX	XX	XX	14

The sites that had the greatest total richness across the four years included Front Porch (medium-use) and Dinosaur Rock (high-use) at 14, Bear Creek Spire (low-use) at 16, and Der Zerkle (high-use) at 19 (Table 4). Interestingly, the four sites with the highest overall richness either are far from the main Mesa Trail (Front Porch) or they have frequent seasonal closures. The last three cliffs sampled had a moderate total richness, with Red Devil (medium-use) having a total richness of 12, and Lost Porch (medium-use) and Der Freischutz (high-use) at 13 (Table 4).

South Ridge had the lowest total richness across the four years (6) and housed only *M. lucifugus* across all four years (Tables 4 and 5). In addition to the little brown bat, only in 2015 was there evidence of *E. fuscus* and *M. ciliolabrum* using this site as a roost. This suggests to me that this site is primarily used by *M. lucifugus*. Similarly, Veranda had *M. lucifugus* roosting in its crevices for all four years of this study and *M. ciliolabrum* was also recorded at this site the first three years (in addition to being visually seen in a crevice in 2014). Interestingly, in the fourth year of study, *M. ciliolabrum* was not at this location, and was replaced by *E. fuscus*. For all four years of this study, Veranda had only two species of bat at most.

Red Devil (total richness of 12) always had three species of bat roosting in its crevices across the four years of this study, but again, only *M. lucifugus* was observed there every single year (Table 5). I also recorded calls of both *M. ciliolabrum* and *M. volans* at this site three of the four years, and *E. fuscus* was observed two of the four summers. Similarly, at Lost Porch (total richness of 13) only *M. lucifugus* was found roosting there every year, but *M. ciliolabrum* and *M. volans* were recorded there three of the four years (Table 5). *E. fuscus* was also observed at this location two of the four

summers, and I also recorded *M. evotis* at this site in 2017. At Der Freischutz (total richness of 13), both *M. lucifugus* and *M. volans* were recorded at this site every year, and *E. fuscus* was observed two of the four years (Tables 4 and 5). I also recorded *M. ciliolabrum*, *M. volans*, and *M. thysanodes* at this location, but based on the scarcity of their presence, these three species may not be consistently using this cliff as a roost. Interestingly, the species richness of Der Freischutz remained at three species of bat until 2017 when a seasonal closure was added to the location (Table 4).

Front Porch (total richness of 14) had both *E. fuscus* and *M. lucifugus* roosting in its crevices all four years (Table 5). In addition, *M. volans* and *M. ciliolabrum* were recorded at this cliff site three of the four years, causing this site to have between three and four species of bat every year (Tables 4 and 5). At Dinosaur Rock (total richness of 14) both *M. lucifugus* and *E. fuscus* were found roosting all four years, and *M. ciliolabrum*, *M. volans*, and *M. thysanodes* calls were recorded from emerging individuals two of the four years (Table 5). This site also had a steady increase in bat richness over the four-year period until 2017. During this year a seasonal closure was lifted, and that year neither *M. ciliolabrum* nor *M. volans* were found at Dinosaur Rock (i.e. richness decreased after the removal of the seasonal closure, Table 4).

Bear Creek Spire (total richness of 16) housed both *M. lucifugus* and *M. ciliolabrum* all four years, and the calls of *M. volans* and *M. thysanodes* were recorded from emerging bats at this site for three of the four years (Tables 4 and 5). I also recorded *E. fuscus* and *M. evotis* at this location, but only for one year each. This cliff is another site that had a seasonal closure for the first three years of this study that was removed early during 2017 because raptors did not nest at this site. The lift of the

seasonal closure corresponds to a slight drop in bat richness at Bear Creek Spire, with the loss of *M. volans* during this fourth year.

Of the nine sites sampled between 2014 – 2017, Der Zerkle consistently had the highest richness of bats, with 19 over the four-year period (Table 4). This site was the only one sampled where all six species of cliff-roosting bats were recorded; however, not every species was found there every year. Both *M. lucifugus* and *M. thysanodes* were observed emerging from this site each year, and *E. fuscus*, *M. evotis*, and *M. volans* were recorded three of the four years (Table 5). Lastly, *M. ciliolabrum* was also found at this cliff, but only during two of the four years. Interestingly, this is also the only cliff-site that had a seasonal closure every single year of this project, due to the population of *M. thysanodes* that was already documented in this location.

After standardizing the number of species found emerging at each site by the surface area of the cliff observed, there was no difference in bat richness based on the use-level categories of OSMP ($F_{2,105} = 2.29$, $p = 0.106$). However, if the sites are rearranged into un-climbed, lightly climbed (1 – 100 climbers per year), and heavily climbed (>100 climbers per year) based on my time-lapse photography, then we see that sites without rock climbing had the greatest bat diversity per square meter of rock (0.0031), followed by cliffs that are lightly climbed (0.00204), and finally those that are heavily climbed (0.0019; $F_{2,105} = 7.25$, $p = 0.0011$). Similarly, the Shannon-Wiener Index for the biodiversity of roosting bats was not different among the OSMP use-levels ($F_{2,35} = 0.12$, $p = 0.887$); but, this index was significantly different when the data were re-organized based on my time-lapse photography ($F_{2,35} = 12.7$, $p < 0.0001$), with the sites without any rock climbing having the highest Shannon Index value (1.03), followed by

sites with high frequency of rock-climbing (0.65), then low rock-climbing traffic (0.39). The evenness of bat species was highest on cliffs without rock climbing (0.75), followed by cliffs with low frequency of rock climbing (0.72) and lastly cliffs with high levels of rock climbing (0.68); however, these differences were not significant ($F_{2,35} = 0.43$, $p = 0.65$; Fig. 16). Interestingly, as the frequency of rock climbing increased, so did the relative abundance of *M. lucifugus*, a species notorious for being more tolerant of anthropogenic disturbance. On average, this species of bat made-up 36% of all roosting bats in cliffs without any rock climbing, and 48% and 55% of all roosting bats in low-use and high-use cliffs, respectively ($F_{2,60} = 3.37$, $p = 0.04$; Fig. 16).

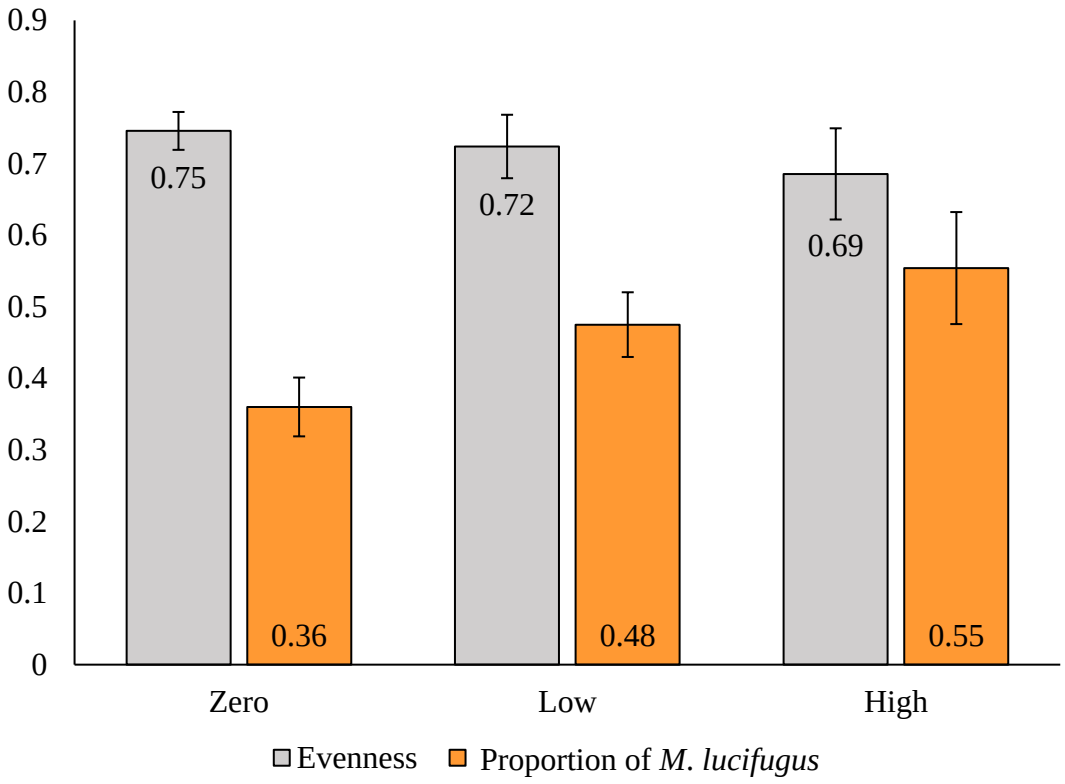

Fig. 16.—Evenness of bat species roosting in cliffs with zero rock climbing, low-frequency of climbers, and high-frequency of climbers, as well as the proportion of *M. lucifugus* at each of these cliff types, a species more-tolerant of human disturbance.

Presence of Bat Roosts on the Nine Cliff Sites

Between 2014 and 2017, bats were observed emerging from multiple crevices on Dinosaur Mountain (Fig. 17), where I located 32 roosts among the nine rock-climbing sites sampled where multiple bats were seen emerging from the cliff (Figs. 18 – 26). Although there were many locations where a single bat would exit a cliff, the only roosts analyzed in this study were those that had multiple individuals, and therefore could potentially be maternity colonies.

The number of roosts on these cliffs varied between two and six, with most sites having three roosts that housed multiple bats. After standardizing the number of roosts per site by dividing this value by the surface area of the cliff observed, the number of bat roosts was significantly higher in areas of moderate climbing according to both the OSMP's categorization of use-levels and the photo-based categories established in this study ($F_{2,105} \geq 4.98$, $p \leq 0.009$). This is likely because these cliffs have more-appropriate angles, heights, and crevice heterogeneity than some of the sites without rock climbing (e.g. South Ridge). It is also possible that the moderate level of disturbance at these sites relative to those with heavy rock-climbing facilitated the increased number of bat roosts.

Many of the bat roosts were located high on the surface of the cliff, with the exception of those individuals that emerged from the talus slopes. Interestingly, the only year I found bats roosting low on the cliff faces of Dinosaur Mountain was during a seasonal closure. Every year after that (when I observed the cliff outside of the seasonal closure time) I did not observe bats emerging from low areas on the cliff face, which may be due to the frequent scrambling by humans over the lower portions of the faces of Dinosaur Mountain.

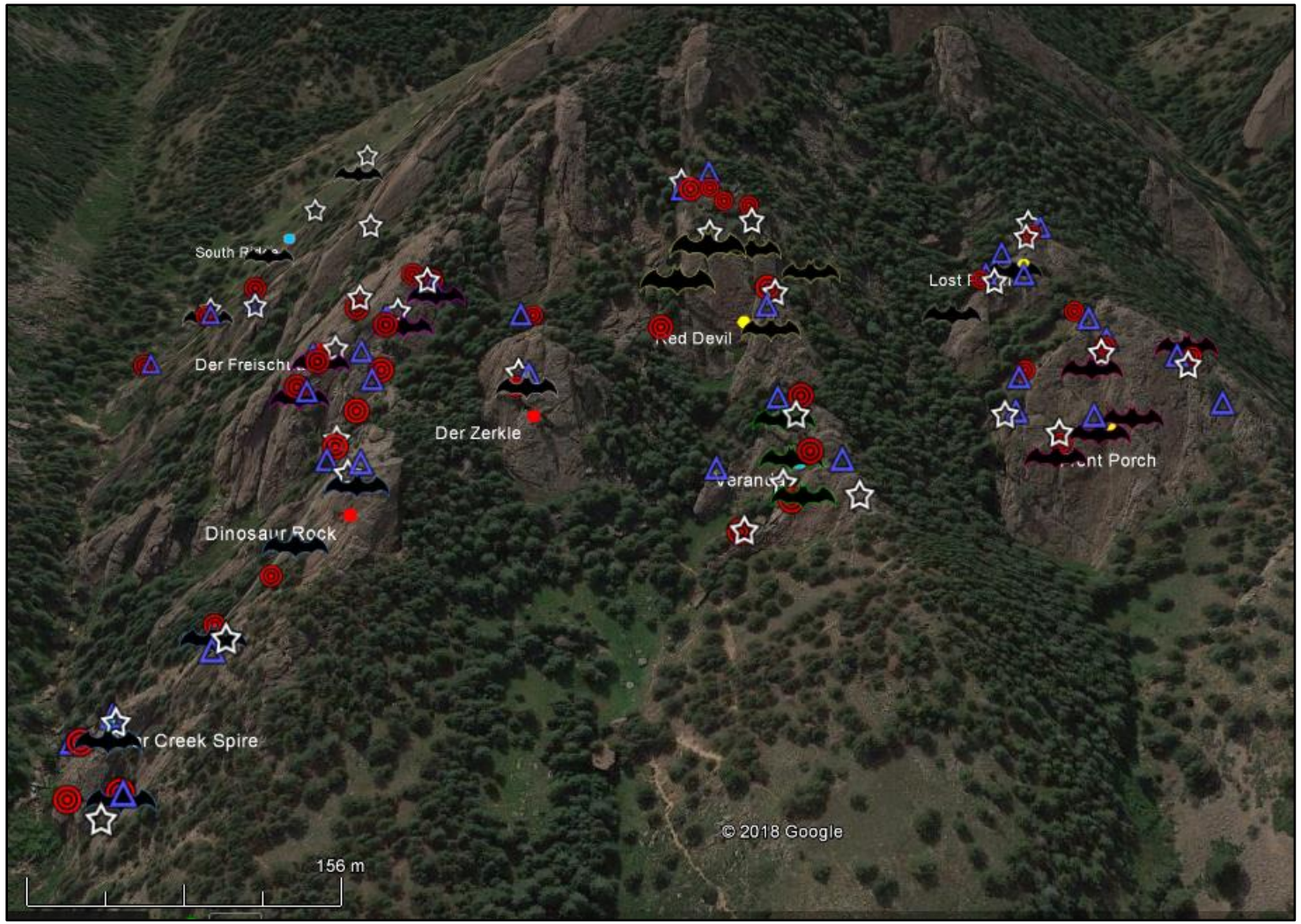

Fig. 17.—Distribution of bat roosts seen on Dinosaur Mountain: bat symbols (2014), white stars (2015), red bulls-eyes (2016), purple triangles (2017). Image taken from Google Earth.

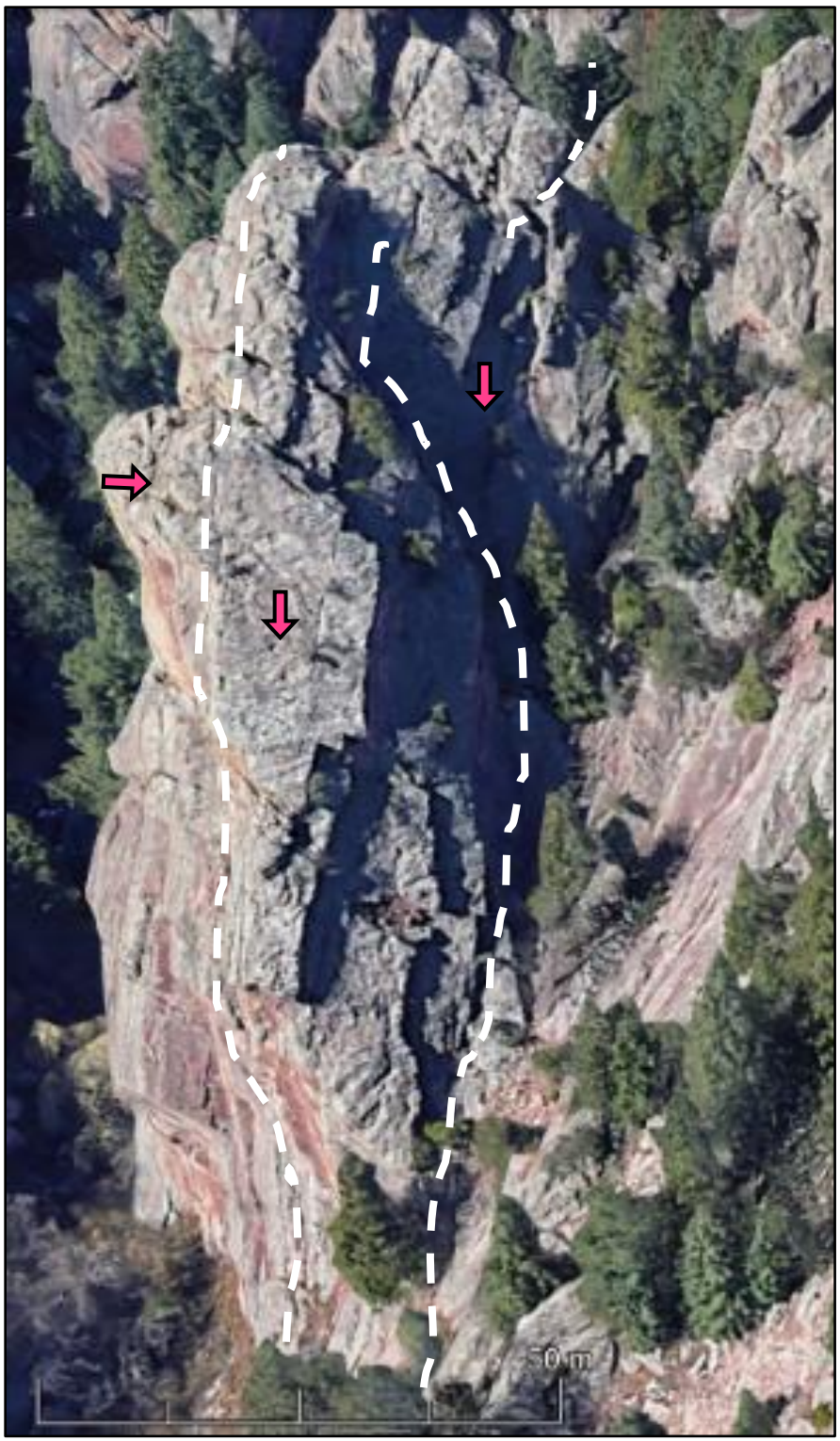

Fig. 18.—Approximate locations of the three bat roosts (arrows) found on Bear Creek Spire (OSMP low-use), as well as the approximate location of established climbing routes (dashed white lines).

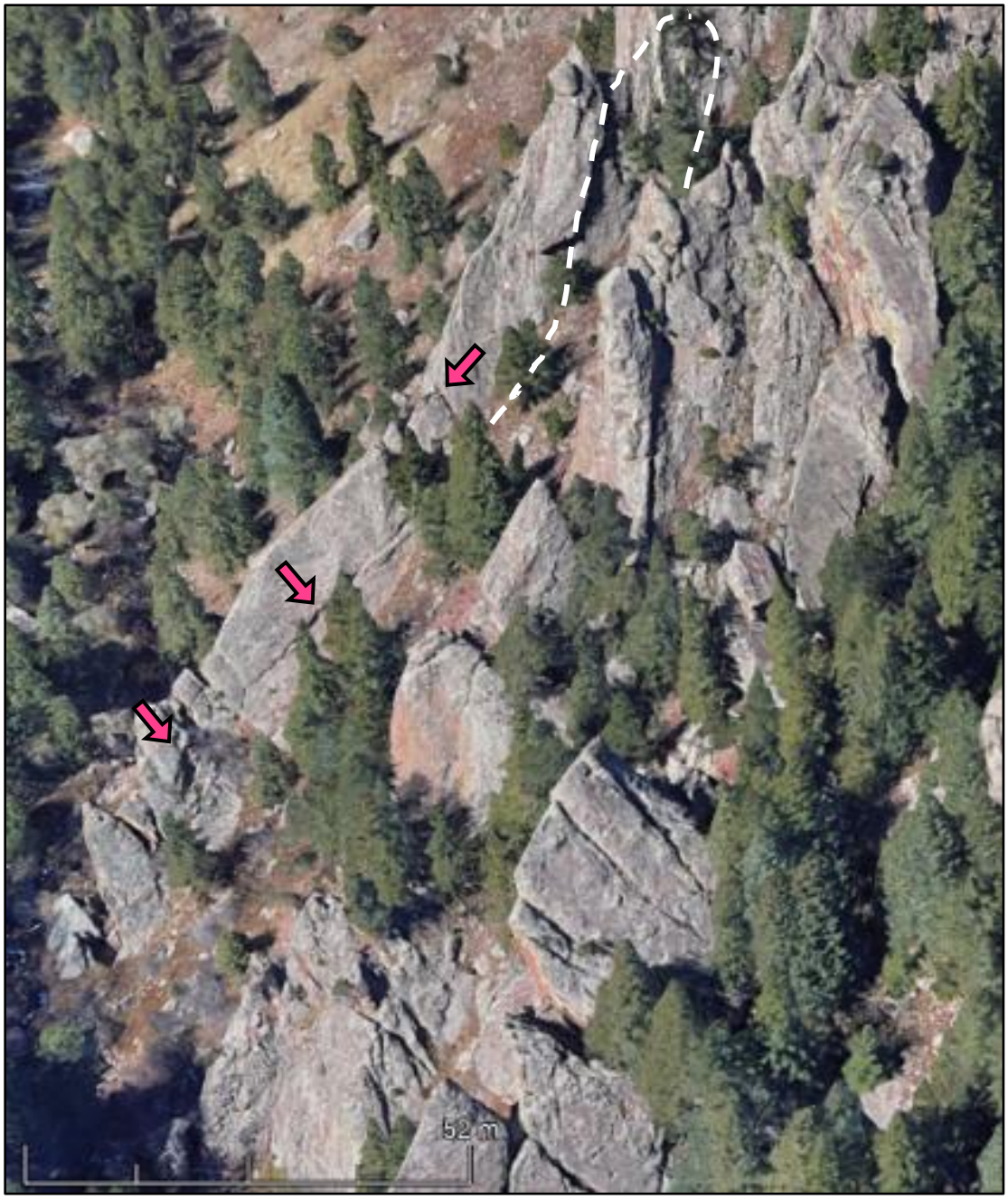

Fig. 19.—Approximate locations of the three bat roosts (arrows) found on South Ridge (OSMP low-use), as well as the approximate location of established climbing routes (dashed white lines).

Fig. 20.—Approximate locations of the three bat roosts (arrows) found on Veranda (OSMP low-use), as well as the approximate location of established climbing routes (dashed white lines).

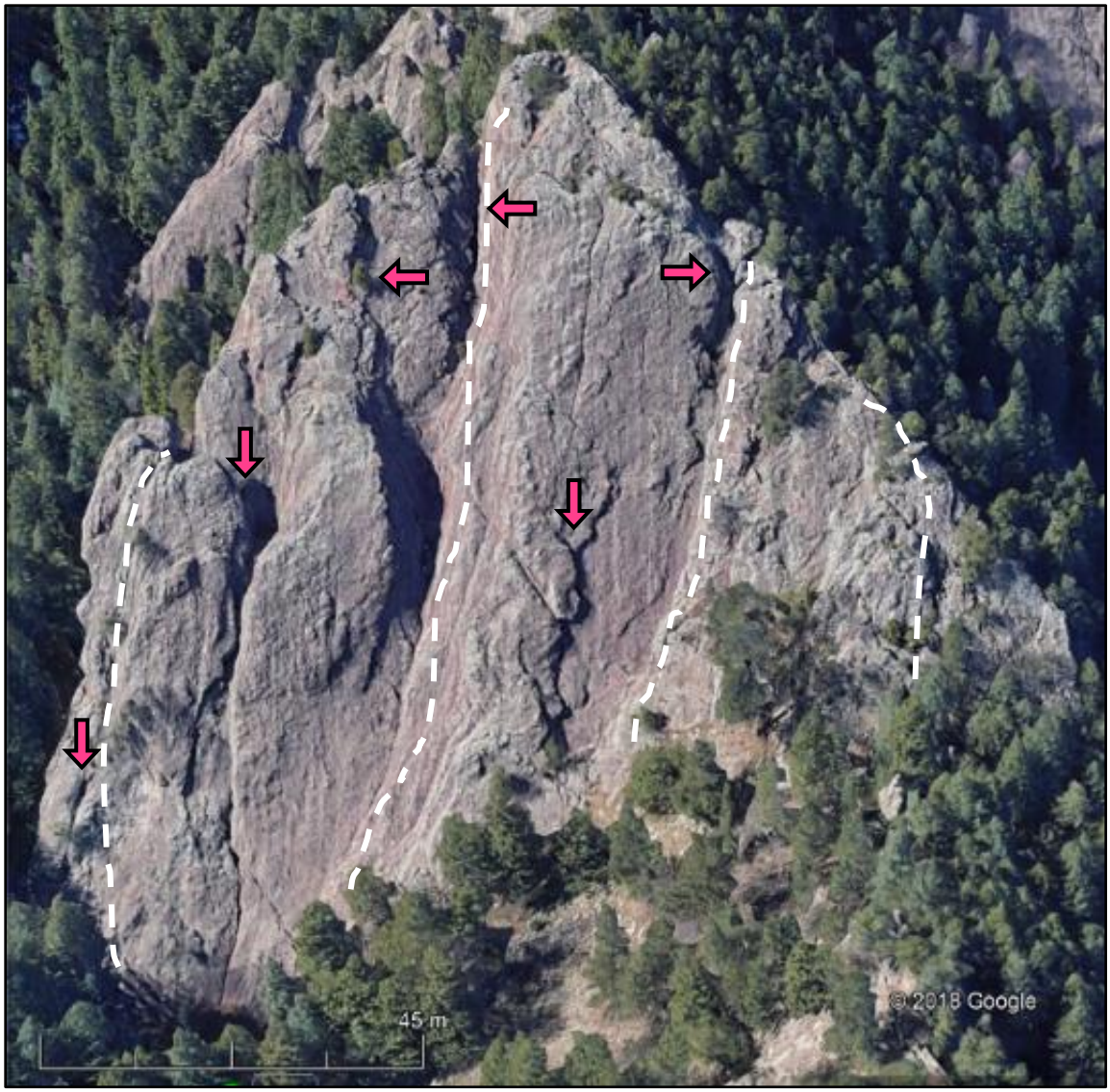

Fig. 21.—Approximate locations of the six bat roosts (arrows) found on Front Porch (OSMP medium-use), as well as the approximate location of established climbing routes (dashed white lines).

Fig. 22.—Approximate locations of the three bat roosts (arrows) found on Lost Porch (OSMP medium-use), as well as the approximate location of established climbing routes (dashed white lines).

Fig. 23.—Approximate locations of the three bat roosts (arrows) found on Red Devil (OSMP medium-use), as well as the approximate location of established climbing routes (dashed white lines).

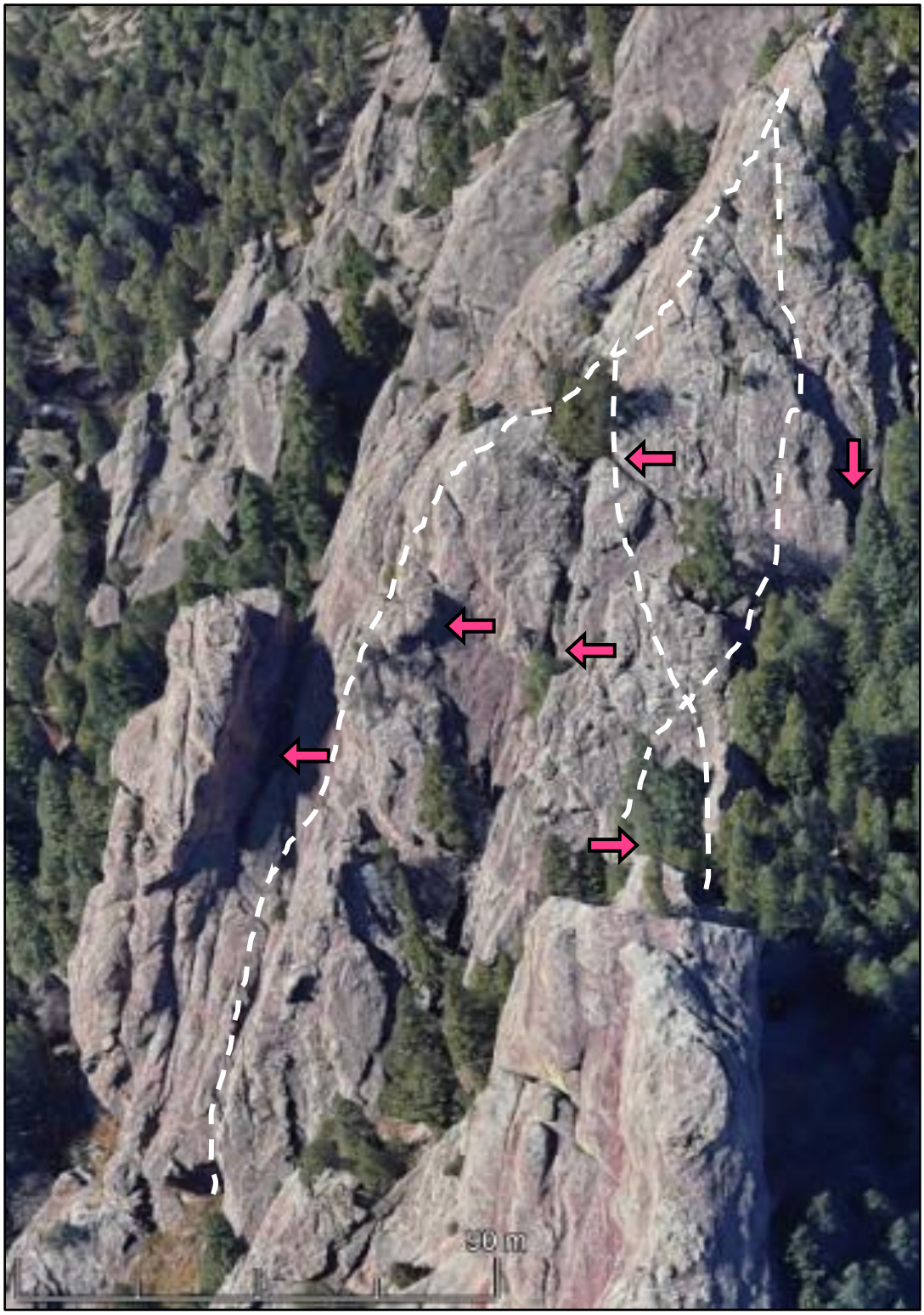

Fig. 24.—Approximate locations of the six bat roosts (arrows) found on Der Freischutz (OSMP high-use), as well as the approximate location of established climbing routes (dashed white lines).

Fig. 25.—Approximate locations of the two bat roosts (arrows) found on Der Zerkle (OSMP high-use), as well as the approximate location of established climbing routes (dashed white lines).

Fig. 26.—Approximate locations of the four roosts (arrows) found on Dinosaur Rock (OSMP high-use), as well as the approximate location of established climbing routes (dashed white lines).

Bat Activity by Year and Levels of Rock-Climbing

Between 2014 and 2017 I visually observed 3,652 bats emerging from the cliffs on Dinosaur Mountain, in addition to 4,334 bats foraging in the areas near the cliffs (Fig. 27). The number of emerging bats was significantly lower in 2014 compared to all other years ($F_{3,104} = 4.9$, $p = 0.003$; Figs. 27 and 28), and the number of foraging bats did not differ among years ($F_{3,104} = 0.93$, $p = 0.43$; Fig. 27). However, it is important to note that many emerging bats continued to forage near their roosting cliff before leaving the area for the night, so the same bat may have been counted as both an emerging and a foraging bat. In addition, the same individual bats were likely counted emerging multiple times, as the same site was observed for three consecutive field nights. Therefore, when considering only the maximum number of emerging bats over the three-night observation period each year, ca. 1,848 bats were observed emerging from the cliffs on Dinosaur Mountain from 2014 – 2017 (Fig. 29).

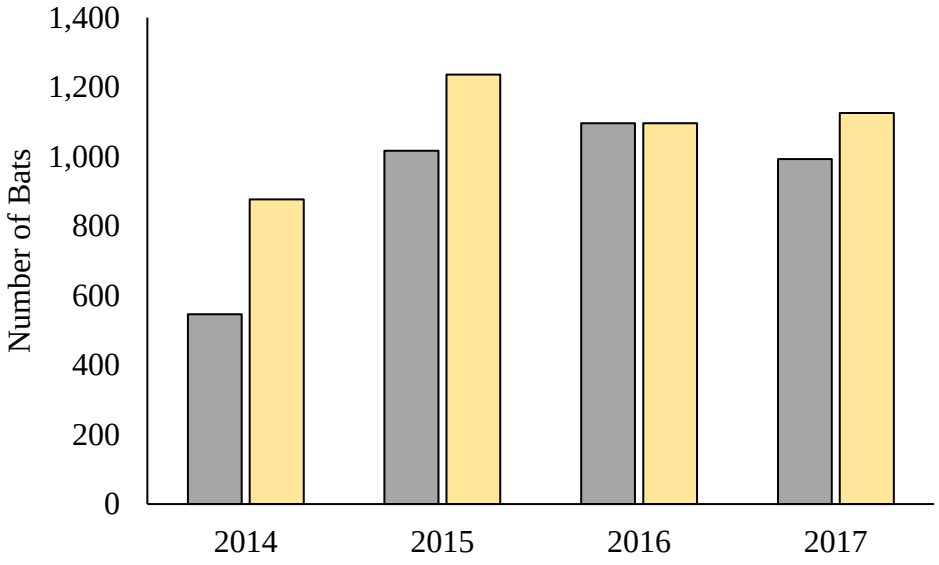

Fig. 27.—Total number of emerging (grey) and foraging bats (yellow) observed on Dinosaur Mountain across all four years of this study.

When comparing the maximum number of emerging bats across years, emergence activity was highest in 2016 but was only significantly higher than the number of bats observed in 2014 ($F_{3,32} = 3.73$, $p = 0.02$; Fig. 28). Interestingly, of the three cliff sites that had an increase in the number of roosting bats in summer 2017, two of them also had seasonal closures for rock climbing that season (Der Zerkle and Der Freischutz; Fig. 29).

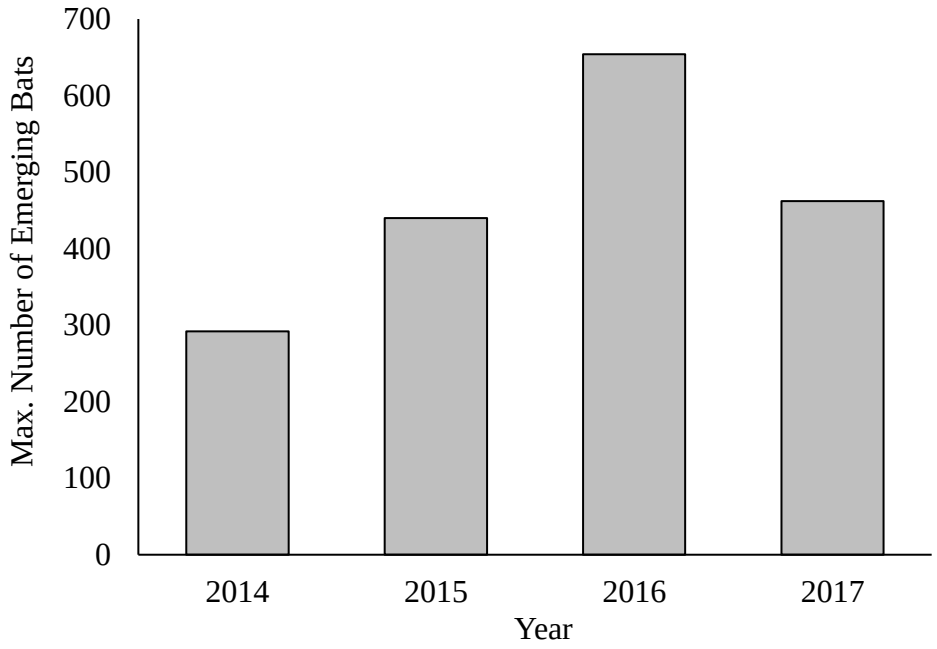

Fig. 28.—Maximum number of emerging bats observed (summed across the nine sites) during each field season from 2014 to 2017.

Bat activity varied across the nine sites (Figs. 29 – 34); however, based on the use-levels used by OSMP, the number of roosting bats between cliff sites did not vary among levels ($F_{2,105} = 2.87$, $p = 0.06$; Figs. 28 and 30). Interestingly, when comparing the number of emerging bats among use-levels based on the time-lapse photography gathered from this study, the number of roosting bats was highest in cliffs with zero rock climbing (0.031 bats/m^2) compared to sites with low levels (0.0253 bats/m^2) and high levels (0.0169 bats/m^2) of rock climbing ($F_{2,105} = 7.25$, $p = 0.0011$; Figs. 31 and 33). Furthermore, when examining the number of roosting bats among the rock-climbing use-

levels obtained from my time-lapse photography, sites with zero rock climbing had the greatest number of bats across all four years of this study (Fig. 34).

Similarly, when the sites were re-organized based on the level of rock climbing observed through the time-lapse photography of this study, sites with no rock climbing had a significantly greater number of foraging bats (0.046 bats/m^2) in the area relative to sites with low (0.023 bats/m^2) and high levels (0.019 bats/m^2) of rock climbing ($F_{2,105}$ = 8.67, p = 0.0003). Interestingly, using an ANOVA with the original categorizations by OSMP yielded a significant, but a much weaker difference among climbing levels, but suggested that foraging activity of bats was significantly lower in areas with high levels of rock climbing (0.016 bats/m^2) relative to sites with zero (0.039 bats/m^2) and low levels (0.032 bats/m^2) of rock climbing ($F_{2,105}$ = 5.36, p = 0.006). Both these results suggested that the foraging activity of bats is lower in areas with high anthropogenic disturbance.

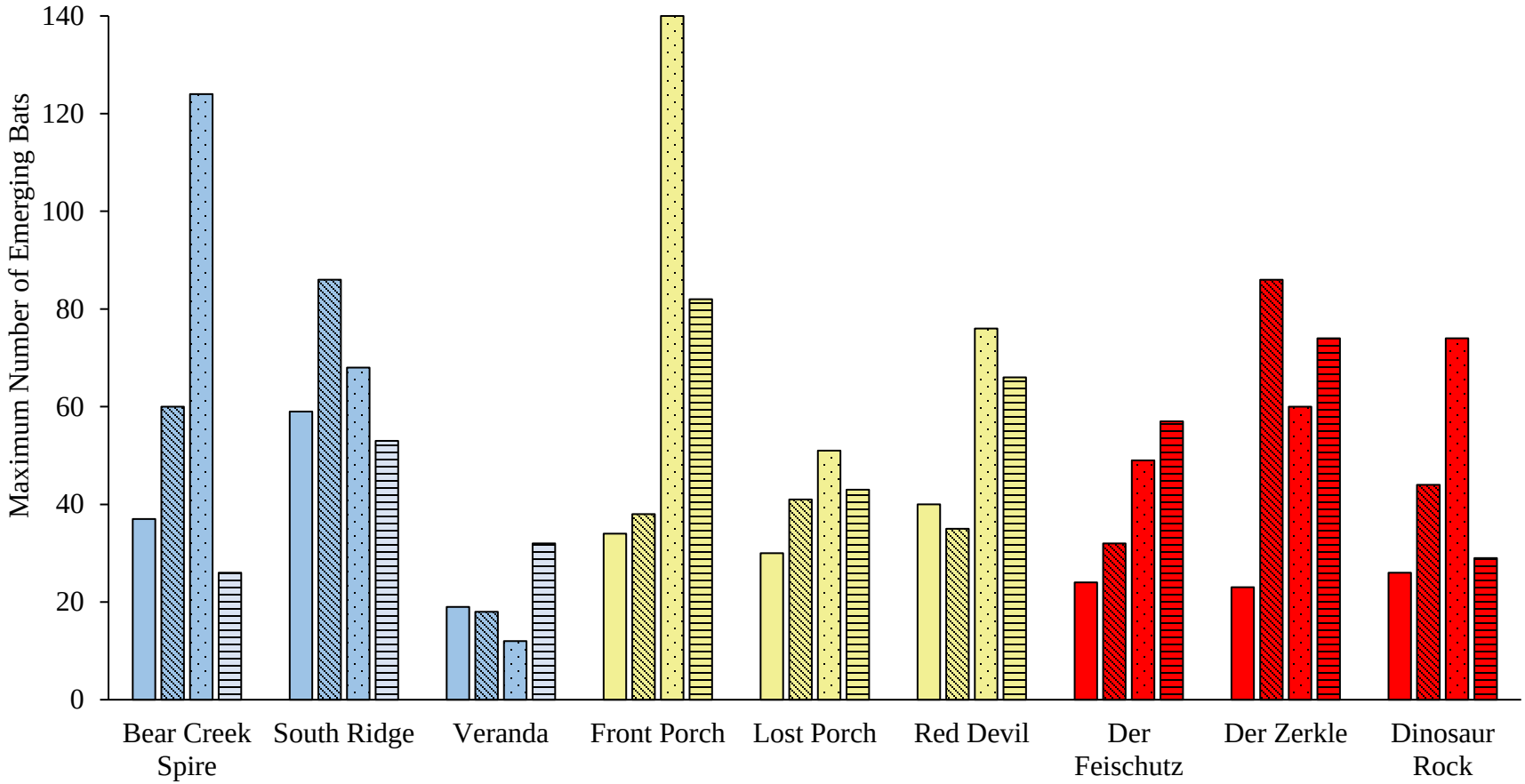

Fig. 29.—Maximum number of emerging bats seen (over the three-night observation period) at each of the nine rock-climbing sites sampled over the last four years (solid bars = 2014, diagonal lines = 2015, dotted bars = 2016, horizontal stripes = 2017). Blue = low use, yellow = medium use, red = high use by rock climbers.

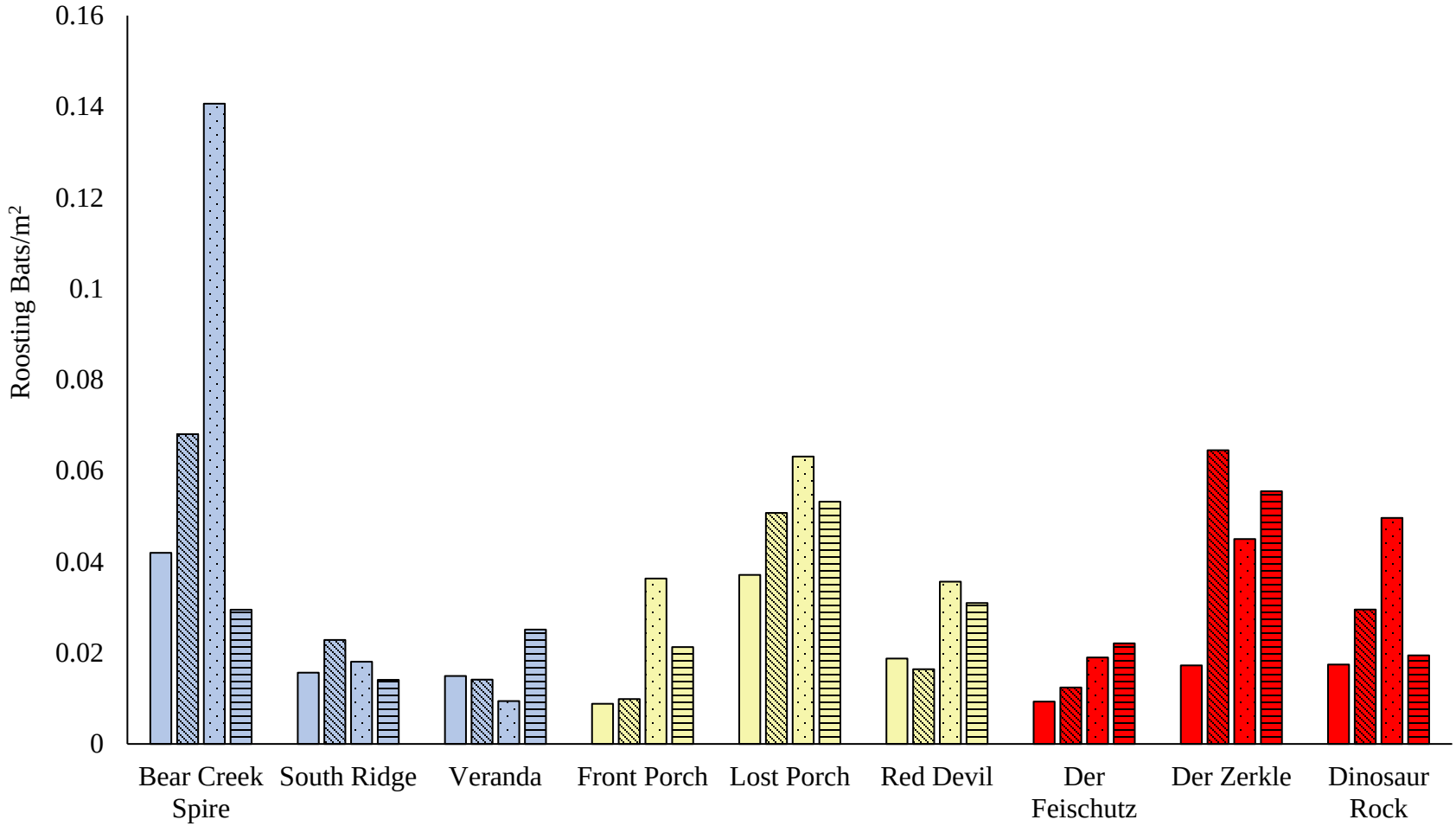

Fig. 30.—Number of roosting bats per square meter, based on OSMP use-levels, with blue being low use, yellow being medium use, and red being high use. Solid bars are data from 2014, diagonal stripes are from 2015, dots are from 2016, and horizontal stripes are from 2017. Note the lack of pattern among use levels.

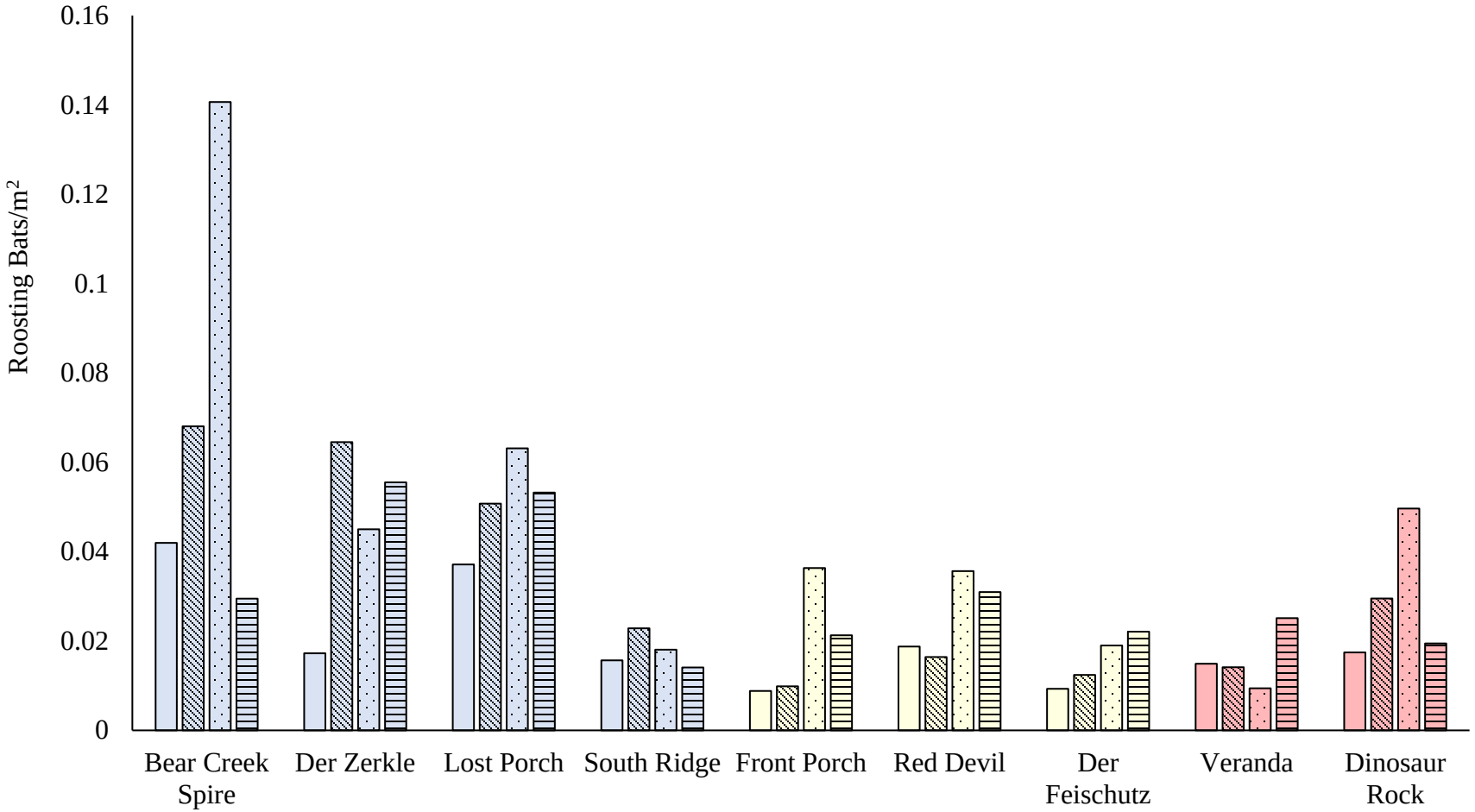

Fig. 31.—Number of roosting bats per square meter, based on the time-lapse photography categorization of use-levels, with blue = zero rock climbing; yellow = low rock climbing (<100 climbers/year), and red = high rock climbing (≥100 climbers/year). Solid bars are data from 2014, diagonal stripes are from 2015, dots are from 2016, and horizontal stripes are from 2017. Note the greater number of bats in (most of) the sites where rock climbing was not recorded on the time-lapse cameras.

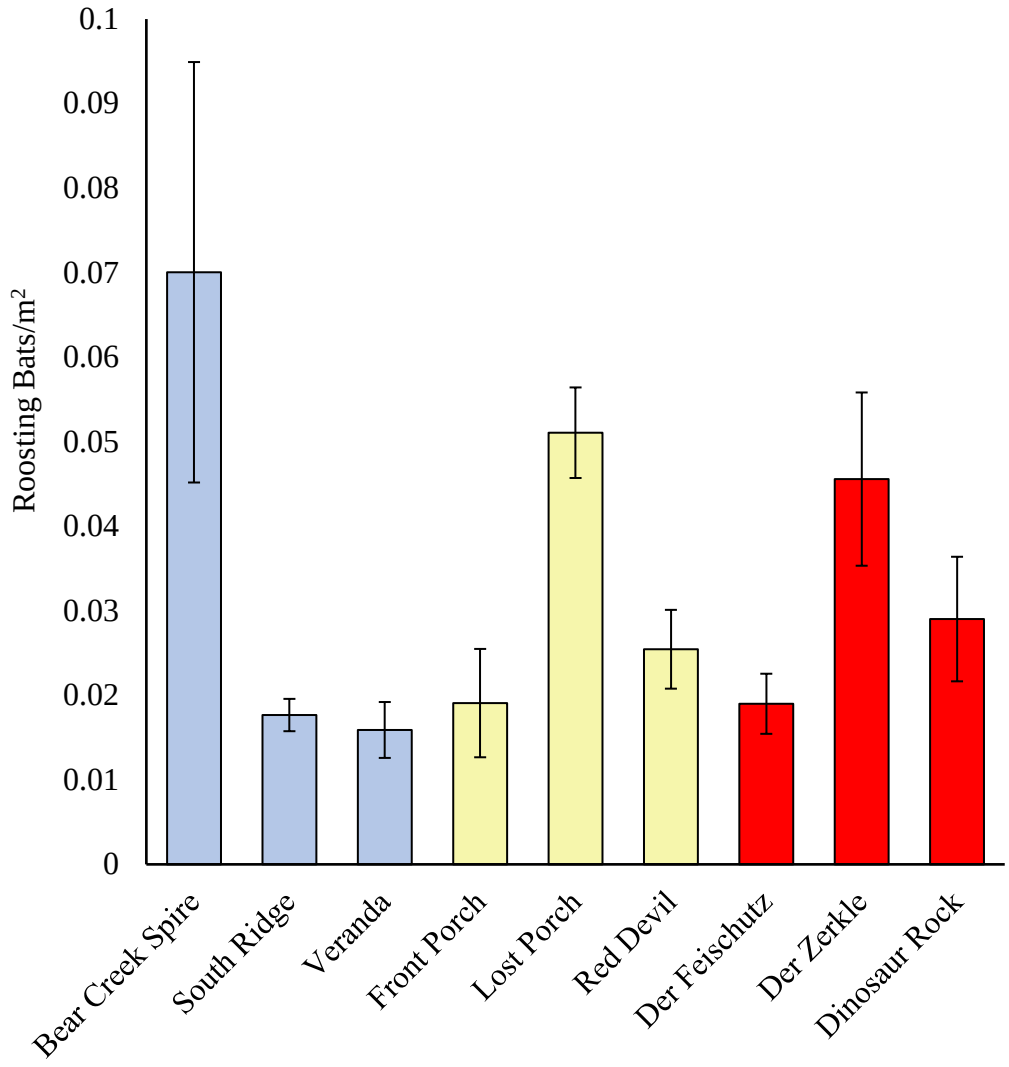

Fig. 32.—Average number of roosting bats/m^2 based on OSMP levels of rock climbing.

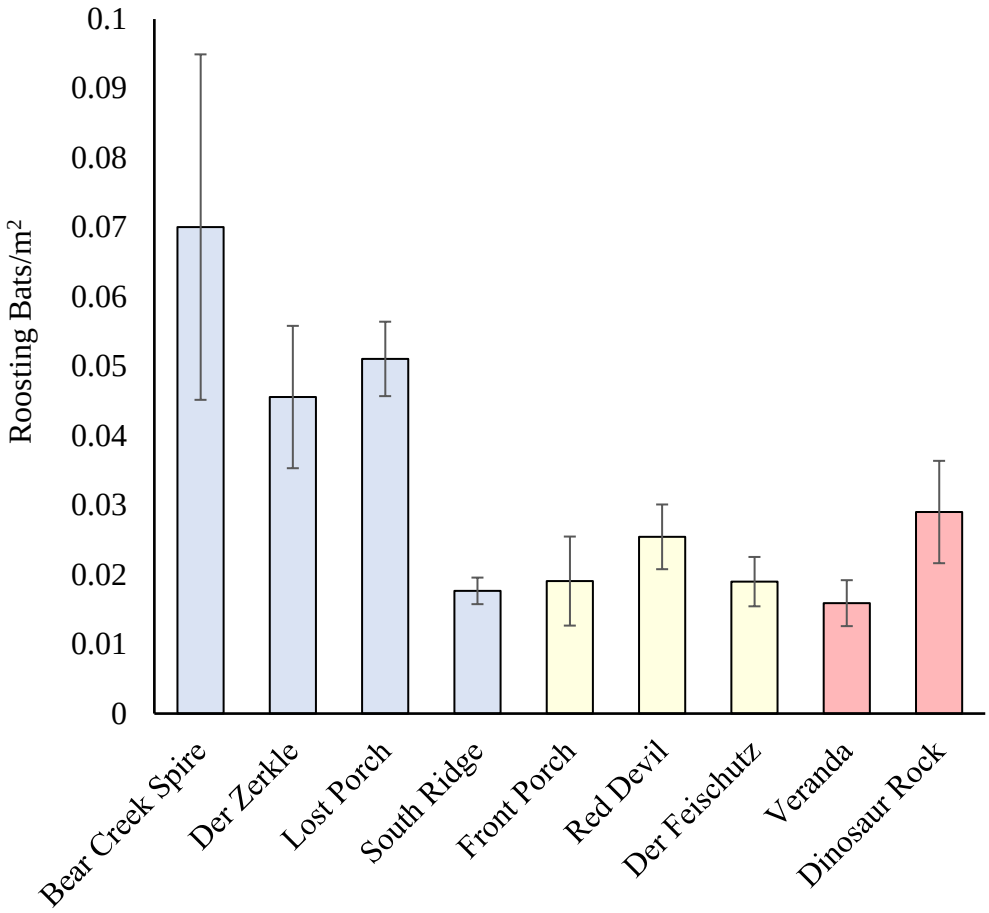

Fig. 33.—Average number of roosting bats/m^2 at all sites based on the rock-climbing use-levels acquired from the time-lapse photography from this study. Note that (most of) the sites without any rock climbing had a greater number of roosting bats across all four years of this study.

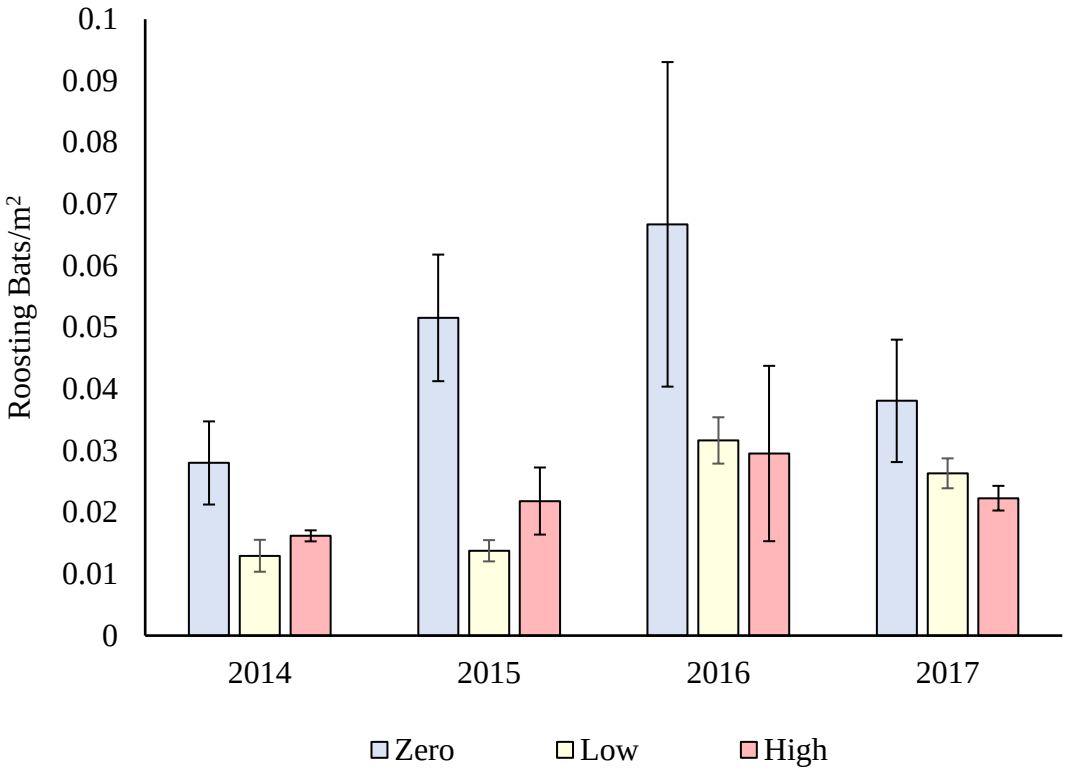

Fig. 34.—Average number of roosting bats/m^2 (across all four years) based on the rock-climbing use-levels acquired by the time-lapse photography.

Bat Activity by Site

Bear Creek Spire is a site that was categorized as low-use by OSMP, which was corroborated with the time-lapse photography in this study, where I found no rock climbers at this location. On this cliff, there were consistently three roosts, two of which are located high-up (≥ ca. 60% up the cliff) on the eastern face, while the third was found on the northeastern corner of the cliff, where it meets an angled slope of loose boulders that lead to Dinosaur Rock (Fig. 26). I recorded echolocation calls of *M. lucifugus* and *M. ciliolabrum* every year, and three of the four years I found evidence of *M. thysanodes* and *M. volans*. This site also had the highest (average) number of roosting bats per unit area out of all the sites sampled (Figs. 30 – 34), all of which may be due to the seasonal closures that have been placed on this cliff during the summers 2014 – 2016 (Table 4).

Lost Porch is a site that is categorized by OSMP as medium-use, but I was unable to find any evidence of rock climbing at this site based on my time-lapse photography. This site consistently had three roosts, one located at the top of the spire on the northern portion of the rock (where the majority of the bats were observed), one on the sloping eastern face of the cliff, and another along the south-eastern rim of the site (Fig. 21). This site housed *M. lucifugus* all four years, and *M. volans* and *M. ciliolabrum* were recorded at Lost Porch three of the four years. In addition, *E. fuscus* and *M. evotis* were also recorded at this location, but not as frequently. This site had the second-greatest number of roosting bats per square-meter (despite lacking a seasonal closure) across the four years (Figs. 30 – 34), which is likely attributed to the fact that this site is the most isolated of the nine sampled.

Der Zerkle is another site where I found no evidence of rock climbing on the eastern face of the cliff, due to a seasonal closure of this aspect to protect a maternity colony of *M. thysanodes*. However, this cliff did have a very high frequency of rock climbing on its western face, giving it a categorization of high-use by OSMP. Two roosts were found at this location. The first roost was already known to OSMP, where the majority of bats emerge from Der Zerkle near a large crux about halfway up the rock that runs horizontally across its face. The second roost of Der Zerkle contained significantly fewer bats, and was located near the top of the cliff, between its middle and left-hand "fingers" (when facing the eastern side of the rock; fig. 25). This site consistently had the highest richness of bats among the nine cliffs, where it houses a colony of threatened *M. thysanodes*. In addition to the fringed myotis, Der Zerkle also contained *M. lucifugus* all four years of this study, and *E. fuscus*, *M. evotis*, and *M. volans* were recorded here three

of the four years, and *M. ciliolabrum* was found just two of the four years (Table 5). Der Zerkle had the third-highest number of roosting bats per unit area, which was likely influenced by the re-occurring seasonal closure (Figs. 30 – 34; Table 4).

South Ridge is another site on which I was unable to find evidence of rock climbing, and this site was also classified as low-use by OSMP. I was able to locate three roosts at this location, all of which were near the top of the rock along is eastern surface (Fig. 18). This site had the fewest number of bats of all the sites without rock climbing, and it also had the third-fewest roosting bats per unit area of all nine sites (Figs. 30 – 34). Although this site has relatively little anthropogenic disturbance (despite lacking seasonal closures and being near main trails), it only consistently housed one species of bat, *M. lucifugus*, with only a single year (2015) when *E. fuscus* and *M. ciliolabrum* were also recorded at this location. Given the short cliffs and shallow aspects of South Ridge, I would presume the limited bat activity at this site is due primarily to inadequate roosting conditions within the rock itself more than anthropogenic activity.

Front Porch is the largest rock sampled during the study; therefore, it was no surprise that it had the greatest number of total roosts (six) and maximum number of emerging bats (Figs. 21 and 29). However, when the surface area observed was taken into account, the number of roosting bats per square-meter at Front Porch was near the median value (Figs. 30 – 34). This location was categorized as medium-use by rock climbers (100 – 500 climbers per year) by OSMP, whereas my time-lapse photography suggests this site might be climbed less frequently than that (ca. 35 climbers per year), which is why I have re-categorized it as low rock-climbing activity (Table 3). At this site I recorded calls from *E. fuscus* and *M. lucifugus* every year, as well as *M. volans* and *M.*

ciliolabrum for three out of the four years, suggesting stable populations of these four species at this site (Table 5). Although it has only a moderate number of bats per unit area, Front Porch may be an important rock to manage on OSMP, due to the large population of bats found at this location, as well as the consistent richness of bats housed in this rock, including *M. volans*, which was relatively uncommon on the mountain. All roosts at this site were located high on the rock, far above from where I recorded humans scrambling on the surface of the rock (Fig. 21).

Der Freischutz is a site that has been categorized as high-use by OSMP; however, I was only able to find low levels of rock climbing at this location with my time-lapse photography (Table 3). This site was the second-largest rock observed, and it had a total of six roosts on its surface, where I consistently recorded echolocation calls of *M. lucifugus* and *M. volans*, and *E. fuscus* two of the four years (Table 5). I also recorded calls from *M. ciliolabrum*, *M. evotis*, and *M. thysanodes* one year (Table 5). Most of the roosts were located high on the rock's eastern face, with the exception of one roost that was found in the large boulders of Der Freischutz, near where it meets Dinosaur Rock, where the majority of *M. volans* were found (Fig. 24). Der Freischutz had the fewest number of roosting bats per square-meter out of all nine sites, which may be affected by the large amount of human foot traffic that passes by this rock as people hike to Mallory Cave (Figs. 30 – 34).

Red Devil is classified as medium-use by OSMP (100 – 500 climbers per year), but according to my time-lapse photography this site was of low-use by climbers (ca. 52 climbers per year; Table 3). This site had three roosts, one of which was found at the top of the southeast corner of the rock, while the other two were about halfway up the eastern

surface of the cliff (Fig. 23). Red Devil had the median number of roosting bats per square-meter, and it consistently housed three species of bat, although the species composition varied among years (Table 5). *Myotis ciliolabrum* and *M. volans* were recorded at this site three of the four years, and *E. fuscus* was found at Red Devil two of the four years (Table 5).

Veranda is categorized as low-use by OSMP (presumably due to its shallow angle), but during my time-lapse photography, it actually had the second-highest level of human climbing (i.e. scrambling) of the nine sites (Table 3). Veranda had a total of three roosts, all of which were found near the top of the eastern faces of the rock (Fig. 20). At this site I consistently recorded *M. lucifugus* all four years, and *M. ciliolabrum* three of the four years (Table 5). This location had the second-fewest bats per square-meter, which is likely due to a combination of the heavy human traffic and shallow angle of this cliff (Figs. 30 – 34).

Dinosaur Rock is classified as high-use by OSMP, which was corroborated with my time-lapse photography that found this site to be the most heavily climbed of the nine sites sampled (Table 3). I found four consistent roosts on Dinosaur Rock, two of which were located near the top of the southeastern face of the cliff, high above where I recorded people scrambling the rock (Fig. 26). The other two roosts were found on the talus slope of the southeastern face of Dinosaur Rock, which is an area less-travelled by humans. At this site, only the disturbance-tolerant *E. fuscus* and *M. lucifugus* were recorded all four years, but *M. ciliolabrum* and *M. volans* were recorded from the talus slopes two of the four years, and *M. thysanodes* was also heard at this site for two years, and this species may be roosting in the crevice located at the top, southwestern corner of

the rock (Fig. 26). Despite the high level of human disturbance at this site, Dinosaur Rock still had a moderate number of bats per square-meter, which may be attributed to the seasonal closures that were placed on this site for three of the four years due to raptor nesting (Figs. 30 – 34; Table 4).

Rock Climbing and Lichen Biodiversity

Photographs of lichens along and away from rock-climbing routes were taken at five of the nine cliffs: Der Zerkle, Dinosaur Rock, Veranda, Der Freischutz, and Front Porch. South Ridge, Bear Creek Spire, and Lost Porch were not photographed due to the lack of evidence of rock-climbing paths on the rock, and Red Devil was not photographed because the climbed area was inaccessible to the researchers. I found at least six different lichens on the cliffs sampled on Dinosaur Mountain (Figs. 35 – 40), which were identified to the lowest taxonomic level possible using A Rocky Mountain Lichen Primer (Corbridge and Weber 1998).

Among these species was a pale-green crustose lichen (with black apothecia) with variable thickness and lumpiness, which was most likely *Aspiclia* sp., one of the most ubiquitous lichen genera of granite rocks (Corbridge and Weber 1998; Fig. 35). Another lichen commonly found on Dinosaur Mountain was a pale-green foliose lichen with broad lobes, a green underside, and no apothecia; therefore, this lichen is most likely *Flavoparmelia caperata*, or the common greenshield lichen (Fig. 36). This species is very common, but it most frequently grows on the surface of vertical cliffs (Corbridge and Weber 1998).

A different lichen found throughout the cliffs of Dinosaur Mountain was another foliose lichen, with large pale greenish-grey lobes and a dark underside, which was

common to areas where water would run-off the cliff-face, and based on its morphology, this lichen is most likely *Parmelia sulcata* (Corbridge and Weber 1998; Fig. 37). An additional species of lichen found was a yellow-rust colored crustose lichen that formed relatively small patches on the rocks. This lichen is most likely a *Candelariella* sp., which is the most common genus of yellow crustose lichens, most of which grow on rocks (Corbridge and Weber 1998; Fig. 38).

I also found two most crustose species on the cliffs of Dinosaur Mountain included two more crustose species. One species was medium-grey in color, with clusters of variable sizes and shapes, and based on its morphology it is likely *Rhizocarpon* sp., a salt-and-pepper lichen that is very common to granite rocks in the Rocky Mountains (Corbridge and Weber 1998; Fig. 39). The other species was a dark-brown/black crustose lichen found in small clusters; although I was not able to confidently identify this species using the Rocky Mountain Lichen Primer, online photos (from Lichenportal.org) supported this lichen to be *Staurothele* sp. (Fig. 40).

Fig. 35.—Crustose lichen, most likely *Aspicilia* sp., that is common to the cliffs on Dinosaur Mountain.

Fig. 36.—Foliose lichen, most likely the common greenshield lichen (*Flavoparmelia caperata*), common to the cliffs on Dinosaur Mountain. These foliose lichens were much less common along climbing routes.

Fig. 37.—Loose foliose lichen with black underside, likely *Parmelia sulcata*, found along the cliffs where water often falls down the walls.

Fig. 38.—Yolk-yellow lichen common to the cliffs on Dinosaur Mountain. Based on its color and crustose growth, this is most likely a *Candelariella* sp.

Fig. 39.—Grey crustose lichen common to the cliffs on Dinosaur Mountain. Based on its color and crustose growth, this is most likely a *Rhizocarpon* sp.

Fig. 40.—Black crustose lichen common to the cliffs on Dinosaur Mountain. Based on its color and crustose growth, this may be a *Staurothele* sp.

The average richness of lichens along rock-climbing paths was 3.8 species, while the average richness of these same organisms was 4.8 species at un-climbed locations ($U = 493$, $z = 4.24$, $p < 0.0001$; Fig. 41). Furthermore, the Shannon-Wiener Index for

lichens was significantly lower along climbing routes (0.83) than unclimbed areas (1.25; $t = 3.18$, $p = 0.001$). I observed that foliose lichens and mosses were extremely rare within the quadrats along climbing routes, presumably because they are easily dislodged from their rocky substrates. As expected, the percent cover (*i.e.* area/abundance) of lichens on these cliffs was significantly higher in areas where humans were not found to climb (85.36%) relative to climbing routes (26.68%; $t = 5.54$, $p < 0.0001$; Fig. 41).

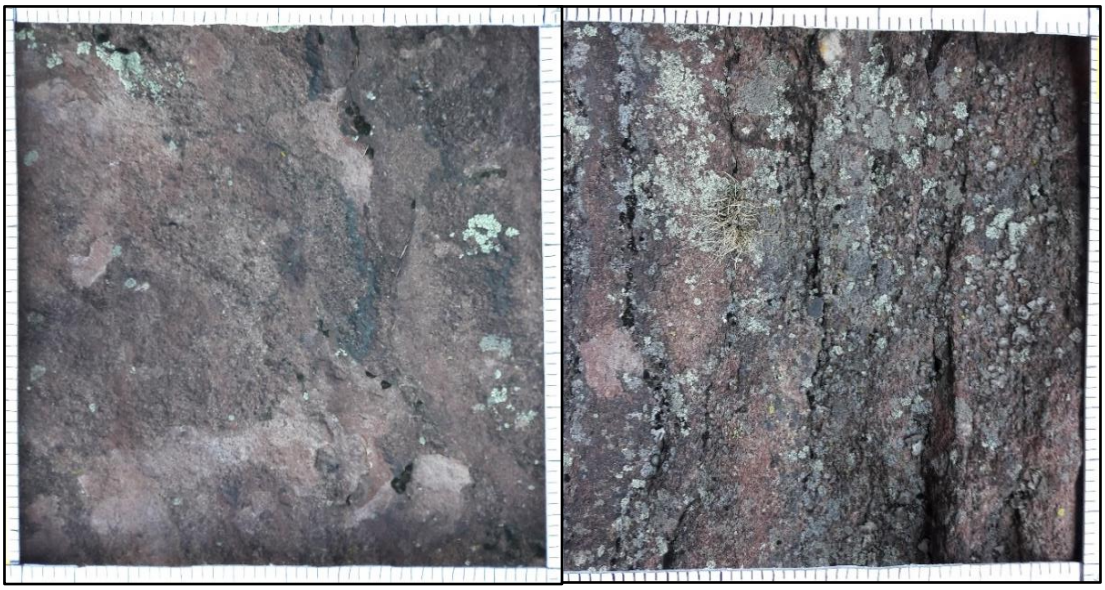

Fig. 41.—Photographs of two quadrats taken at Veranda, one along a rock-climbing route (left) and another in a non-climbed location on the cliff (right). Note the significant difference in the percent cover of lichen between these two areas of the same cliff (photos by A. K. Wilson).

When correlating the frequency of rock climbing with the percent cover of lichens along climbing routes, the data suggest a negative correlation between these two variables ($R^2 = 0.72$; Fig. 42) that is not linear, but rather logarithmic. Although this is a strong correlation, I believe this relationship would be even greater if more than five sites were included in the regression analysis. Interestingly, there is also a strong negative correlation between the percent cover of lichen and average number of rock climbers on unclimbed areas of the cliffs as well ($R^2 = 0.82$; Fig. 43). This suggests that people may

be climbing on these “unclimbed” areas of the cliff as well, but less frequently, and therefore were not recorded on the time-lapse photography. The percent cover of lichen measured was used in the multivariate analysis of this study. For the sites where no rock climbing was observed, the percent cover was estimated based on the linear regression established in this study (Fig. 43).

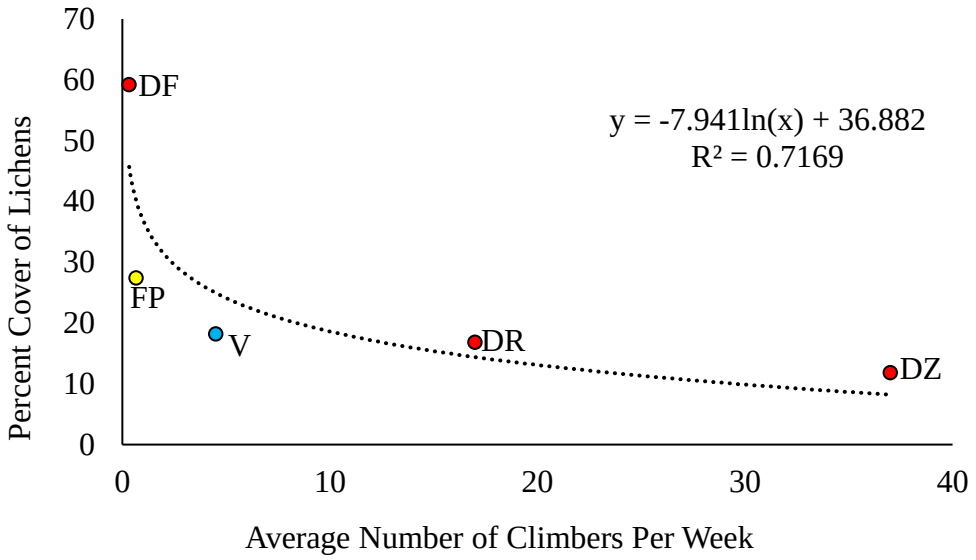

Fig. 42.—Logarithmic regression between the frequency of rock climbing and the percent cover of lichen along rock-climbing routes. DF = Der Freischutz, FP = Front Porch, V = Veranda, DZ = Der Zerkle, and DR = Dinosaur Rock.

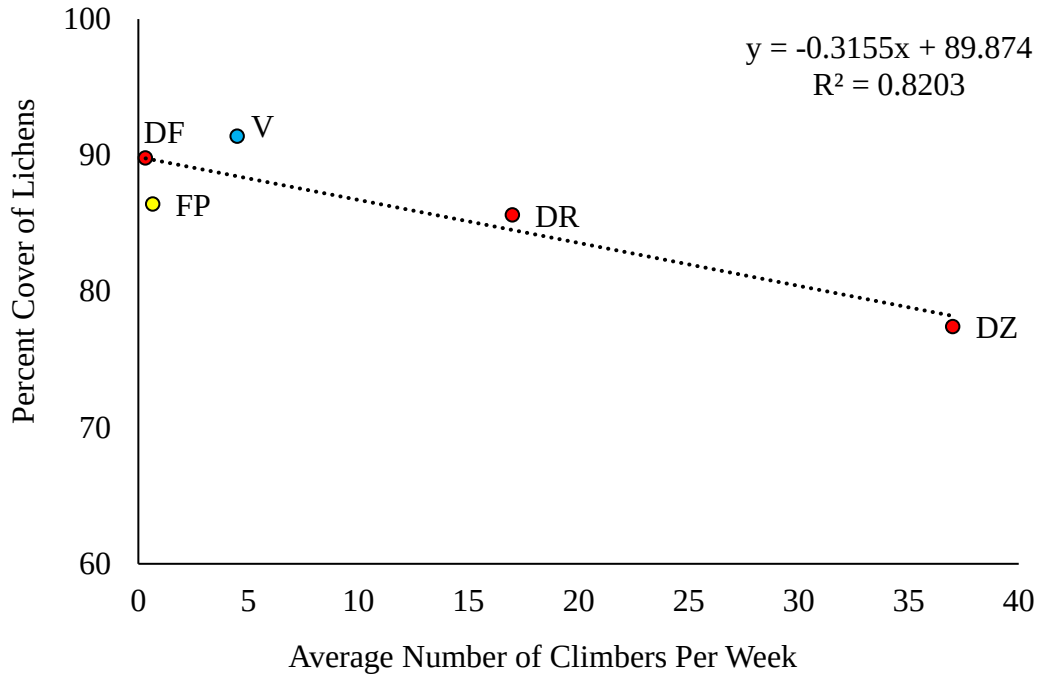

Fig. 43.—Linear regression between the frequency of rock climbing and the percent cover of lichen on un-climbed areas of the cliff. DF = Der Freischutz, FP = Front Porch, V = Veranda, DZ = Der Zerkle, and DR = Dinosaur Rock.

Multivariate Analysis of Rock Climbing Characteristics and Bat Activity

To assess the impacts of multiple rock-climbing characteristics on measures of bat activity on Dinosaur Mountain, I used Canonical Correspondence Analysis (CCA). This multivariate statistic llows one to separate multiple dependent and independent variables and to correlate these multiple variables in a two-dimensional map. For my CCA I used the climbing characteristics (independent variables) seasonal closures, average route difficulty, number of climbing routes, percent traditional routes, trail traffic, climbers per week, and lichen cover to explain the bat activity (dependent variables) on Dinosaur Mountain including bat richness, number of roosts, quantity of roosting bats, total foraging bats.

The CCA map revealed that 92.12% of the variation found among the different dependent variables can be explained by the climbing characteristics used, with the first axis explaining 69.89% of this variation (eigenvalue: 0.024; Fig. 44). Both seasonal closures and percent lichen cover had a positive correlation with the bat activity with respect to the first axis, suggesting that these two variables have a strong positive relationship with bat activity at these sites (Fig. 44; Table 6). All other climbing characteristics had a negative association with bat activity with respect to the first axis, with climbers per week having the greatest negative association with bat activity (Fig. 44; Table 6).

The second axis of the CCA map explained 22.22% of the variation in the bat-activity data (eigenvalue: 0.009; Fig. 44). Interestingly, 'seasonal closures', 'average route difficulty', and 'percent traditional routes' all had a positive association with bat activity on Dinosaur Mountain (Fig. 44; Table 6). Similar to the first axis, the climbing characteristics 'climbers per week', 'number of routes', and 'trail traffic' all had a negative relationship with bat activity at the locations sampled (Fig. 44; Table 6), which are all indirect measures of anthropogenic disturbance.

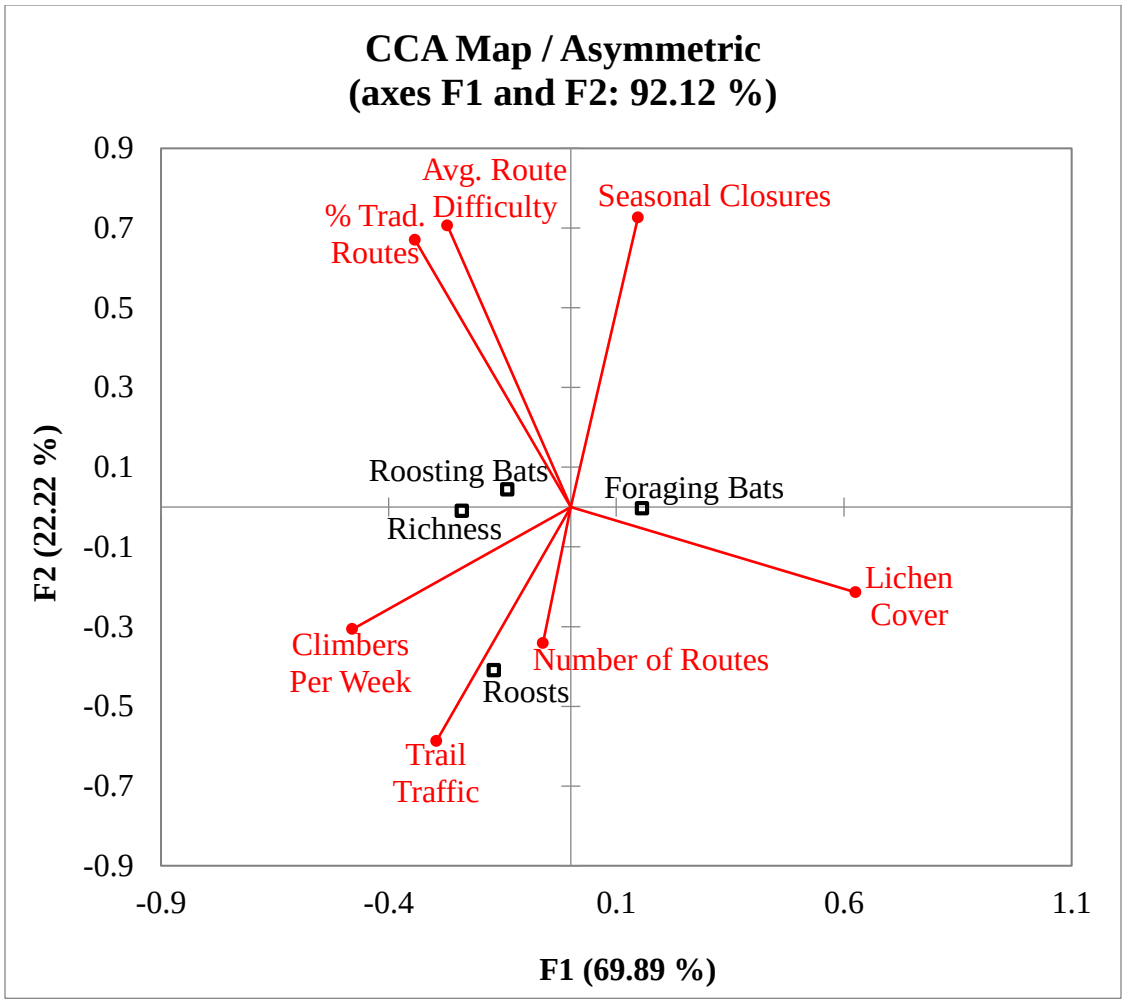

Fig. 44.—CCA map showing the correspondence between the climbing characteristics used (red) with the bat activity (black) on the nine sites sampled on Dinosaur Mountain.

The two climbing characteristics that had a negative correlation with bat activity on the first axis, but a positive relationship on the second axis were ‘average route difficulty’ and ‘percent traditional climbing routes’. These variables likely have a negative correlation on the first axis due to the presence of (any) rock climbing having a negative impact on bat activity, while the type of rock climbing also has an impact. For example, when the climbing routes are more difficult and when there is a greater proportion of traditional climbing routes, there are likely to be fewer rock climbers at those sites; therefore, it makes sense that these variables would first have a negative

relationship with bat activity, but also a positive association as an increase in these climbing characteristics should indicate a decreased level of anthropogenic disturbance. Conversely, the variables ‘climbers per week’, ‘trail traffic’, and ‘number of routes’ all have negative relationships with bat activity on the first axis, as well as the second axis (Fig. 42; Table 6). Therefore, as each of these climbing characteristics increases, we would expect to see an increase in the negative impact of these variables on bat activity (i.e. an increase in ‘climbers per week’ and ‘trail traffic’ would increase levels of anthropogenic disturbance).

With respect to the bat-activity variables, number of foraging bats, quantity of roosting bats, and bat richness were all strongly associated with the correlations of the first axis (Table 7). The number of roosts however, was best explained by the second axis. Therefore, we can see that ‘foraging bats’ and ‘roosting bats’ have a strong relationship with the climbing characteristics lichen cover and seasonal closures (i.e. the presence of closures and more lichen cover is associated with more foraging bats and roosting bats). Conversely, the number of bat roosts is closely associated with the number of climbing routes, which may be due to the cliff-face requirements necessary for both bats and rock climbers (i.e. appropriate slope, height, crevice depth, etc.). Overall, we can see that the richness of bats, as well as the number of roosting and foraging bats have a greater correlation with lower levels of anthropogenic disturbance (i.e. fewer climbers per week and fewer climbing routes), with seasonal closures being the only variable with a positive correlation with bat activity on both axes (Fig. 44; Table 6).

Table 6.—Relative influence of each climbing variable on the two different axes. Note that only 'seasonal closures' has a positive correlative with bat activity on both axes.

Climbing Variable	F1	F2
Lichen Cover	0.626	-0.214
Seasonal Closures	0.148	0.726
Number of Routes	-0.061	-0.341
Avg. Route Difficulty	-0.271	0.706
Trail Traffic	-0.295	-0.587
% Trad. Routes	-0.342	0.670
Climbers Per Week	-0.480	-0.307

Table 7.—Relationship between the various measures of bat activity and the axes produced by the CCA.

Bat Activity	F1	F2
Foraging Bats	0.504	0.001
Roosting Bats	0.349	0.107
Richness	0.100	0.001
Roosts	0.048	0.892

Discussion

Bat biologists have long known that bats roost in the crevices of vertical cliffs, and they appear to be important geological features for bats (Ancillotto et al. 2014; Loeb and Jodice 2018). However, this dissertation is the first long-term study to investigate the impacts of rock climbing on the roosting activity of bats.

Bats, Cliffs, and Rock-Climbing

On Dinosaur Mountain, a property of City of Boulder Open Space and Mountain Parks, I located 32 roosts where multiple bats were seen emerging on the nine cliffs sampled during this study from 2014 – 2017. At these locations, I found evidence of six

species of bat using these nine rock-climbing cliffs as summer roosts (from most to least common): *M. lucifugus*, *M. ciliolabrum*, *M. thysanodes*, *E. fuscus*, *M. volans*, and *M. evotis*. A single *C. townsendii* was observed in a cave-like outcropping three of the four years, but it was not included in the analysis of this study. In addition, I also recorded the calls of three tree-roosting species, *L. borealis*, *L. cinereus*, and *L. noctivagans*, but these species were not included in the analysis, as they do not roost in cliff crevices.

The big brown bat (*Eptesicus fuscus*; Fig. 13) is a medium-sized (14 – 21 g) bat with an overall brown coloration and a keeled calcar (Adams 2003). *Eptesicus fuscus* is tolerant of human activities, and is often commensally associated with people by roosting in barns, attics, bridges, etc. (Adams 2003). Despite their tolerance of humans, big brown bats also roost in rocky outcroppings and crevices. Although males will roost solitarily or form small bachelor colonies of a few individuals during the summer, female big brown bats have been known to form larger maternity colonies of several hundred individuals, where the females leave their pups in the roost while they forage (Fig. 45). The echolocation call of *E. fuscus* is frequency modulated and steeply sweeping (from ca. 70 – 30 kHz) that often has a harmonic (Maxell et al. 2011; Fig. 6). The big brown bat is essentially cosmopolitan in the United States, occurring in deserts, deciduous woodlands, evergreen forests, and scrubland (Adams 2003). In the state of Colorado *E. fuscus* can be found in riparian forests, meadows, aspen woodlands, and ponderosa pine woodlands to name a few (Armstrong et al. 1994). This species tends to emerge near dusk, and it flies relatively low (6 – 10 m), where is aerially hawks insects (primarily beetles) from the air.

Fig. 45.—Neonate big brown bat (*E. fuscus*) found within a crevice on Dinosaur Rock in 2014. Photo was taken by A. K. Wilson after the mother had emerged to forage for the night.

The western small-footed myotis (*Myotis ciliolabrum*; Fig. 13) is the smallest bat in Colorado, weighing just 4 – 6 g. In addition to its small size, this species is recognized by its light-colored, yellowish fur and dark muzzle, ears, and forearms (Halloway and Barclay 2001). *Myotis ciliolabrum* is insectivorous, eating primarily beetles, moths, and lacewings (Freeman 1984) that it grasps from the sky (Adams 2003). This species ranges from southern British Columbia through southern Arizona and New Mexico (Adams 2003). *Myotis ciliolabrum* prefers drier locations such as deserts, badlands, and semi-arid habitats where cliffs and scree fields are available (Halloway and Barclay 2001; Adams 2003). The western small-footed myotis forms summer maternity roosts in talus slopes and cliff crevices in Colorado, where it prefers moderate to low elevations (Armstrong 1994; Adams 2003). This bat emerges just before dark (Fenton et al. 1980), where it flies low, slowly, and with great maneuverability (Norberg and Rayner 1987) near cliff faces (Adams 2003). The echolocation call of this species is frequency modulated, starting

near 80 kHz and abruptly dropping to 40 kHz, often with a characteristic downward tail at the end of the call (Adams 2003; Maxell et al. 2011; Fig. 6)

The western long-eared myotis (*M. evotis*; Fig. 13) is another small (5 – 8 g) insectivorous species that has the longest ears of any myotis in North America (Adams 2003). In addition to its large, dark ears, this species is recognized by its straw-colored fur that is black at the base. *Myotis evotis* inhabits temperate forests from central British Columbia through central Arizona and New Mexico (Adams 2003). This species flies through denser vegetation, where it gleans insects (moths, beetles, flies, true bugs and lace wings) off the surface of leaves and bark (Adams 2003). This bat is found in numerous habitat types (shrublands, semi-arid, subalpine), and in Colorado it is common in ponderosa pine forests (Armstrong et al. 1994). Although this species uses a variety of ecosystems for roosting (bridges, caves, hollow trees, loose bark, etc.), it prefers rocky areas (Solick and Barclay 2006) where colony sizes range from 12 to 30 individuals (Adams 2003). *Myotis evotis* has a steeply sweeping echolocation call (ca. 90 – 30 kHz) that appears almost linear, where it can have up to 100 kHz of bandwidth in just a few milliseconds (Maxell et al. 2011; Fig. 6).

The little brown bat (*M. lucifugus*; Fig. 13) is a smaller bat (7 – 14 g) with brown, glossy fur (Adams 2003). Some of the distinguishing characteristics of this species include the lack of a calcar and long hairs on their feet that extend past the toes (Adams 2003). *Myotis lucifugus* ranges from Alaska to central Mexico where is roosts practically everywhere (in buildings, trees, caves, piles of wood, mines, cliff crevices, bridges, etc.) and its diet consists primarily of midges, but it also contains mosquitos, beetles, craneflies, wasps, and bugs (Adams 2003). This species prefers to forage over water, but

it also exploits open areas and can hunt between the trees (Adams 2003). The echolocation call of this species sweeps from 70 kHz to ca. 30 – 40 kHz and has a duration of at least 7 ms (Adams 2003; Maxell 2011; Fig. 6). Like *E. fuscus*, the little brown bat is also tolerant of human activities, and it often found roosting in buildings occupied by humans. This species also forms maternity colonies that may have thousands of individuals (Adams 2003), and roost availability appears to be the limiting factor for populations of *M. lucifugus* (Fenton and Barclay 1980). Once the most common bat in North America, populations of the little brown bat out east are now being decimated by White-Nose Syndrome, a condition caused by a pathogenic, cold-loving fungus (*Pseudogymnoascus destructans*) that is passed between bats and can be transmitted by humans (Blehert et al. 2009).

The fringed myotis (*M. thysanodes*; Fig. 13) is a smaller species (7 – 14 g) that has long ears and a dark muzzle. In addition to a lighter-brown pelage, this species is easily identified by the series of stiff hairs that extend from the edge of its tail membrane (Adams 2003). The fringed myotis eats beetles and moths that it hawks near the tree canopy using its maneuverable flight (Adams 2003). The echolocation call of *M. thysanodes* has a large bandwidth over a short duration, yielding an almost vertical call that ranges from ca. 85 – 25 kHz that is often associated with harmonics (Adams 2003; Maxell et al. 2011; Fig. 6). *Myotis thysanodes* ranges throughout western North America, from British Columbia to Mexico, although its records in the Rocky Mountains are scattered (Adams 2003). In Colorado, this species roosts in rock crevices, but it has been found to roost in caves, mines, and buildings elsewhere. (Adams 2003). This

species is particularly sensitive to human disturbance, especially near maternity colonies (O'Farrell and Studier 1980).

Finally, the long-legged myotis (*M. volans*; Fig. 13) is a smaller bat (6 – 9 g) that is similar in appearance to *M. lucifugus*, but it can be discerned by its keeled calcar, short toe hairs, and fur that extends to its elbow on its ventral surface (Adams 2003). *Myotis volans* is a direct flier, chasing and consuming moths and other soft-bodied insects (Warner and Czaplewski 1984) over relatively long distances, both through and around the canopy (Adams 2003; Johnson et al. 2007). The echolocation call of this species is frequency modulated and has a diagnostic upward stroke to its call (although this trait is rarely seen), with a bandwidth that ranges from ca. 90 – 40 kHz (Maxell et al. 2011; Fig. 6). This species ranges from southern Alaska to northern Mexico and occurs throughout the Rocky Mountains, where it moves up in elevation at the temperature increases throughout the summer (Adams 2003). The long-legged myotis is found in ponderosa-pine forests, aspen forests, and mountain meadows in Colorado (Armstrong et al. 1994), where it roosts in trees, rock crevices, and rocky cracks near stream banks where maternity roosts are often formed (Adams 2003).

Of the six cliff-roosting species observed, *E. fuscus*, *M. ciliolabrum*, and *M. lucifugus* were recorded at every site; however, only *M. lucifugus* was recorded at all nine sites every single year. These results are not surprising, as *M. lucifugus* and *M. ciliolabrum* were the most commonly recorded species on Dinosaur Mountain, and both *E. fuscus* and *M. lucifugus* are relatively tolerant of human disturbances. The rarest species recorded on Dinosaur Mountain was *M. evotis*, which accounted for 1.6% of the calls (Fig. 15). This bat was recorded from Der Zerkle three of the four years, and was

recorded at Bear Creek Spire, Lost Porch, and Der Freischutz just one year each (Table 5). *Myotis volans* was also among the more-rare species recorded, at just 4.3% of the total calls (Fig. 15). This species was found at Der Zerkle every year of this study, as well as at Bear Creek Spire, Front Porch, Lost Porch, Red Devil, and Der Freischutz three of the four summers, and at Dinosaur Rock for two of the years (Table 5). Lastly, *M. thysanodes* (a state-threatened species), was found at Der Zerkle all four summers (from a previously known roost), but this species was also recorded at Bear Creek Spire three summers, Dinosaur Rock two years, and Der Freischutz one summer. It is possible that the seasonal closures of Bear Creek Spire and Dinosaur Rock influenced *M. thysanodes* to use these cliffs as a roosting location; however future studies should be conducted by OSMP to confirm the presence of this species at these two sites.

The bat richness, number of roosts, and quantity of roosting bats and foraging bats were standardized for the surface area of the cliff observed prior to statistical analyses. I also ran these statistical tests using the rock-climbing use-levels organized by OSMP as well as the use-levels established by the time-lapse photography used during this study. When using the OSMP categorization, I found no significant differences between bat richness, the number of roosts, nor the quantity of roosting bats among use-levels. However, when using the categorization based on my time-lapse photography, bat richness and the number of roosting bats was highest in areas with zero rock-climbing, whereas the number of roosts was highest on cliffs with low levels of rock-climbing. Sites where bat richness was the highest were those that had seasonal closures (Der Zerkle, Bear Creek Spire, Dinosaur Rock), were far from main trails (Front Porch, Lost Porch), and/or had tall vertical slopes (all listed but Lost Porch).

Interestingly, when examining the difference in the number of foraging bats among use-levels, the OSMP categorization found the fewest number of foraging bats in high-use areas, while the categorization based on the time-lapse photography showed the greatest number of foraging bats in areas with zero rock-climbing. Both of these differences suggest that increased anthropogenic disturbance via rock-climbing is negatively associated with the number of foraging bats near the cliffs. Interestingly, when re-categorizing the rock-climbing use-levels based on the time-lapse photography, the number of both roosting and foraging bats was greatest in areas with zero rock-climbing all four years of this study (Fig. 34).

After watching the frames produced by the time-lapse photography, I measured the richness, biodiversity, and percent cover of lichens on the surface of the cliff along and away from rock-climbing paths. On the cliffs sampled for lichens, I identified six species of lichen, and the richness, biodiversity, and percent cover of lichens were all higher in areas where rock-climbing was not recorded. I also found a strong negative correlation between the percent cover of lichen and the number of rock climbers per week. These results are not surprising, given that previous research on other cliffs have found a similar trend (Baur et al. 2007; Adams and Zaniewski 2012; Studlar et al. 2015).

The canonical correspondence analysis (CCA) showed a strong association between the number of foraging and roosting bats with ‘seasonal closures’ and ‘percent lichen cover’ (Fig. 44). The CCA also found that the number of bat roosts was correlated with the number of climbing routes. This may be due to larger rocks having a greater number of routes, and arguably more habitat heterogeneity, which may provide more crevices for suitable roosting. Interestingly, only the variable ‘seasonal closures’ had a

positive correlation to the bat activity variables on both of the principle axes produced by the CCA (Fig. 44). These results suggest that seasonal closures have a positive impact on bat activity at cliff sites, which makes sense, as bat richness and number of roosting and foraging bats were all greater at sites without rock climbing.

During the final analysis of this project, one study was conducted on bat activity along sandstone cliffs in Tennessee, where the eastern small-footed bat (*M. leibii*) has been found roosting (Loeb and Jodice 2018). Loeb and Jodice (2018) compared bat activity between climbed and unclimbed cliffs, but they did not find evidence of rock climbing impacting the richness of these bats, nor the activity of bats. However, this study was just conducted for one summer. In my study, it was essential for me to monitor the cliffs for multiple seasons to identify most (if not all) of the roosts on the cliff-face. For example, I found the fewest number of roosts during my first field season, which was likely influenced by my novice experience, as all subsequent years the number no new roosts were found. Not only that, but environmental conditions across years can impact bat activity, therefore it is imperative that field-based observational studies, such as this one, be conducted for more than one year. Another important factor for future studies to consider is standardizing all of their dependent variables by the surface area of the cliff before determining if any significant differences are seen in the data, as this was essentially in elucidating the impacts of rock climbing on bat activity in this study.

Conclusions

This study was the first to assess the impacts of rock climbing on the activity of bats, and overall, I found evidence that anthropogenic disturbance via rock climbing has negative impacts on bat activity. Because bats play critical roles in their ecosystems, but

are suffering population declines, it is essential that wildlife managers monitor populations of cliff-roosting bats while mitigating high levels of rock climbing where populations of bats are known to roost. For example, the CCA from this study suggests that seasonal closures positively impact bat activity on cliffs in Boulder, Colorado. Therefore, similar restrictions could be implemented on rock-climbing cliffs with roosting bats elsewhere, especially where species of bat that are threatened/endangered, sensitive to disturbance, vulnerable to White-Nose Syndrome, and/or form maternity colonies. The proper management of rock-climbing activity and populations of cliff-roosting bats is attainable, which can allow both humans to enjoy nature, while protecting the essential biodiversity of cliff-face ecosystems.

CHAPTER III

IMPACTS OF BATS ON THE BIODIVERSITY OF CLIFF CREVICES

Abstract

Biodiversity is directly linked to ecosystem functioning, and with the increasing impact humans are having on the planet, biodiversity is declining worldwide. Vertical cliffs are rare ecosystems that can house high biodiversity, which has been attributed to the heterogeneity of the rocky habitat. Because bats roost within the crevices of cliffs, they likely influence the biodiversity of bacteria, fungi, and mesofauna (e.g. small insects, spiders, mites, etc.) within the soils of these cracks by depositing guano and urine, thus providing resources that would otherwise be absent. I used DNA analysis to quantify the biodiversity of bacteria and fungi within the soils of bat roosts and the soils from crevices without bats. I also used visual microscopy to determine the biodiversity of mesofauna within these same crevices. Overall, the biodiversity of bacteria was significantly higher in bat roosts ($t = 2.33$, $p = 0.012$), whereas the biodiversity of fungi was slightly lower in bat roosts ($t = 1.62$, $p = 0.056$). The biodiversity of mesofauna was also significantly lower in crevices without bats ($t = 3.05$, $p = 0.0017$). Bacteria and fungi often have antagonistic relationships in nature; therefore, it is not surprising that fungal biodiversity would be low when bacterial biodiversity is high. Increased bacterial biodiversity may help to cycle nutrients (e.g. nitrogen) that are

essential for the establishment of plants, thus increasing the functionality of these rare ecosystems.

Introduction

Vertical cliffs are found on every continent, and their various cracks and crevices serve as microhabitats within the outcroppings of the rock (Larson et al. 2000). These microhabitats lead to variations in factors such as levels of moisture, exposure to wind, and ambient temperatures among the crevices of a single cliff, which can have major effects on the diversity of plants, animals, fungi, and bacteria. Because of this, many cliffs have relatively high levels of biodiversity in a small area of space (Larson et al. 2000). Although a handful of studies have investigated the biodiversity of plants on cliff faces, no study has yet investigated the biodiversity of bacteria and fungi within the soil of cliff crevices.

Previous investigations have concluded that there is a direct link between biodiversity and ecosystem functions (e.g. primary productivity, nutrient cycling, nitrogen fixation, carbon sequestration, etc.— Tilman et al. 2012; Pasari et al. 2013), with many emergent properties of ecosystems being affected by the loss of just a single species within a community (Norris 2012). Tilman et al. (2012) also found biodiversity to be the strongest driver of ecosystem function over time. Because bird guano was found to influence the biodiversity of microbiota and arthropods within cliff-face substrates (Kolb et al. 2015), it is reasonable to presume that bat guano will also have a positive impact on the biodiversity of microbiota and mesofauna within the cliff crevices.

I examined the relationships among the presence of cliff-roosting bats on the biodiversity of organisms living in the soils of cliff crevices, and hypothesized that the

biodiversity of bacteria, fungi, and mesofauna (e.g. small insects, spiders, mites, etc.) would be higher in bat roosts compared to cliff crevices without bats.

Methods

I estimated how bats influence the biodiversity of the mesofauna and microbiota of cliff-face ecosystems by collecting soil samples from cliff crevices where bats are known to roost (from 2014 – 2016 observations), as well as from nearby crevices where bats were not found roosting. I determined a cliff crevice to have resident bats if at least one of the following criteria were met: 1) at least one bat was seen in the crevice, 2) at least one bat was undoubtedly seen emerging from the crevice, and/or 3) bat guano was found below or in the crevice. Furthermore, I considered crevices failing to meet all three of these criteria as devoid of roosting bats and therefore were the best samples to collect for crevices without bat activity. Although these sampling methods are not randomized, being able to confidently confirm (and access) all the bat roosts found at these cliffs is nearly impossible, so randomizing crevices to sample was not feasible. In an attempt to reduce further bias, soil samples from crevices without bats were collected near (within 5 m of) those cracks with roosting bats, which should help to standardize the impacts of other variables such as elevation, light exposure, moisture availability, etc. on biodiversity values.

From my nine rock-climbing sites, I gathered a small amount (up to 2 ml) of soil from the crevices housing bats (n = 26) and those without roosting bats (n = 26), using pre-sterilized 2-ml Eppendorf tubes. In addition, any fecal pellets or biological debris (e.g. insect carcasses, spider exuviae, etc.) found in the crevices were also gathered in separate Eppendorf tubes at this time. I sterilized all equipment used in the field to

collect soils by spraying 91% ethanol on all exposed surfaces in between samplings. Each sample of soil was placed immediately on ice, and all samples were stored in a -80^{o}C freezer at the University of Northern Colorado until further analyses were conducted (i.e. analysis of microbial DNA, microscopy of mesofauna, and nutrient analysis). In order to limit the amount of contamination in the soil samples, DNA analysis was conducted prior to the microscopy of mesofauna and nutrient analysis (Chapter IV).

Molecular Analysis

From each sample of soil gathered in the field, 0.25 grams were used for the isolation of microbial DNA via a PowerSoil® DNA Extraction Kit (following the manufacture's protocol), in order to analyze the biodiversity of microscopic fungi and bacteria in the dirt via automated ribosomal intergenic spacer analysis (ARISA; Fig. 46). ARISA is a polymerase-chain-reaction- (PCR-) based approach that amplifies the highly variable region of ribosomal DNA (rDNA) found between the 16s and 23s coding regions of the nuclear genome (Schabereiter-Gurtner and Rölleke 2003; Okubo and Sugiyama 2009; Sanschagrin and Yergeau 2014). This technique has been used extensively to analyze the structure of microbial communities; however, ARISA has not been used to analyze biodiversity of cliff soils.

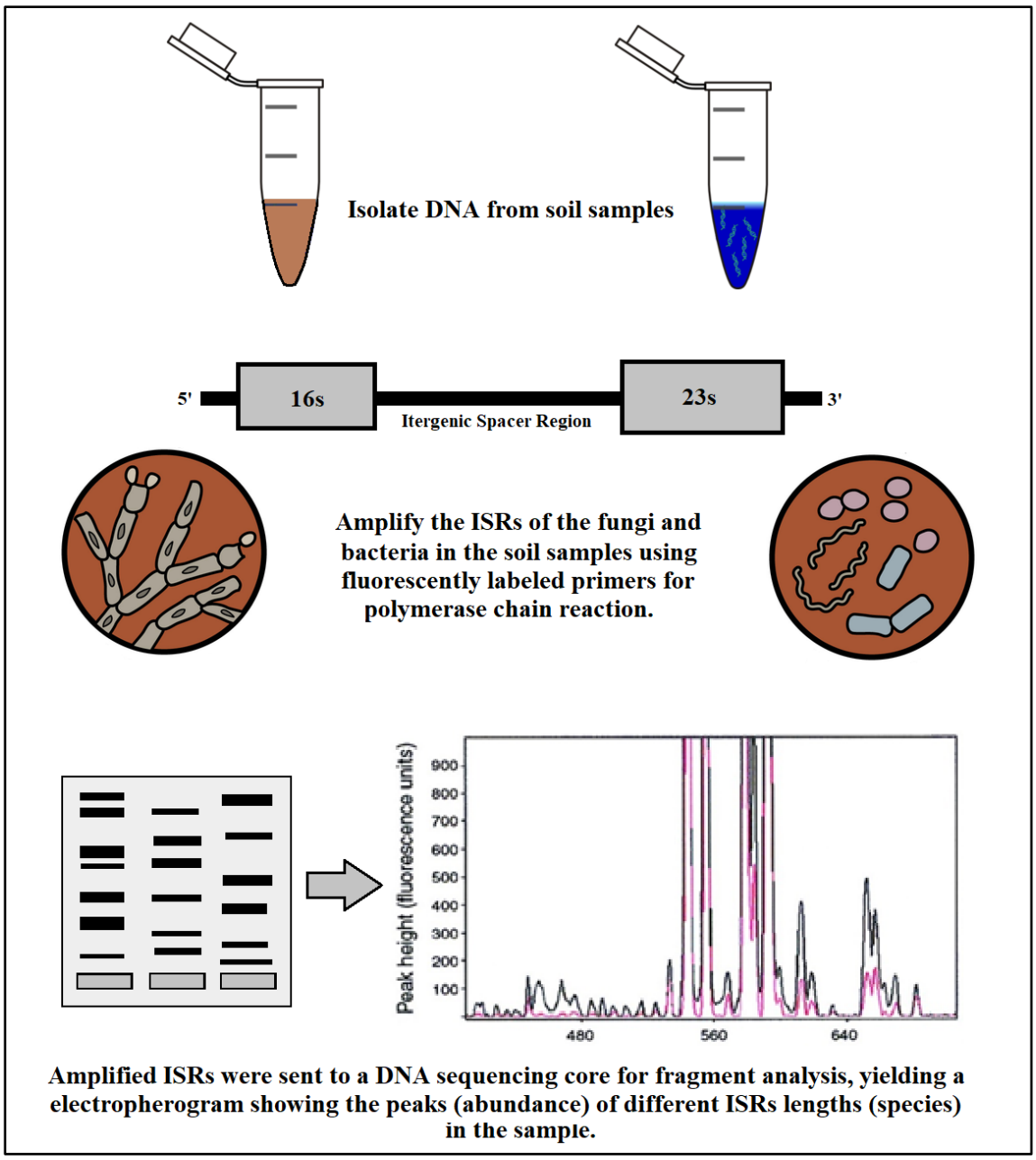

Fig. 46.—Diagrammatic representation of ARISA, including DNA isolation, ISR amplification vis PCR, and fragment analysis (created by A. K. Wilson).

After completing the DNA extraction, samples of DNA were stained with Sybr-green (2 µl of DNA with 2 µl of dye) and were run through a 1% agarose gel (120 volts for 20 minutes) to confirm the presence of isolated DNA. I chose to use the primers from Ranjard et al. (2001) for my ARISA because these primers were used to amplify of both fungal and bacterial DNA within samples of soil with relatively high success. Moreover,

I chose these primers based on their efficacy described by Ranjard et al. (2001), as well as their lower degree of hair-pinning and dimerization compared to the primers used in other publications. These primers were used to amplify the intergenic spacer region (ISR) all species of fungi and bacteria within each sample of soil in separate 21-µl PCRs (Table 8). I used the fluorescent tag 6-FAM (blue) for the fungal primers and the fluorescent tag HEX (green) for the bacterial primers. All conditions for PCR followed a standard protocol used to amplify cpDNA, which was established by M. E. McGlaughlin (pers. comm; Table 9). All PCR products were stained using Sybr-green (2 µl of PCR product with 2 µl dye) and were run through a 1% agarose gel (120 volts for 45 minutes) with a 1Kb-ladder used as a size standard.

Table 8.—Reagents and volumes for polymerase chain reaction for fungal and bacterial ARISA (pers. comm. M. E. McGlaughlin).

Volume	Ingredient
1 µL	Untagged primer (10 µM)
1 µL	Tagged primer (10 µM)
4 µL	Buffer (Promega® 5X Go Flexi)
1 µL	dNTP mix (2.5 mM)
1 µL	$MgCl_2$ (25 mM)
0.3 µL	Taq Polymerase (Promega® Go Flexi)
11.7 µL	dH_2O (for balance up to 20 µL)
1 µL	Extracted DNA (use non-barrier tips!)

Table 9.—Thermocycling conditions for the polymerase chain reaction for ARISA (pers. comm. M. E. McGlaughin).

PCR Stage	Time and Temperature
1) Initial denaturing	5 min. @ 80ºC
2) Continued denaturing	1 min @ 80ºC
3) Annealing	1 min. @ 50ºC
4) Extension	4 min. @ 65ºC
5) Go to '2' x 30 cycles	
6) Final Extension	5 min. @ 65ºC
7) Temporary hold	@ 4ºC

Each sample of DNA that was successfully amplified for the fungal and bacterial ISRs were dried overnight and sent to Arizona State University (ASU) for fragment analysis. This analysis determines the number of nucleotides within a PCR product, which can be used to assess the size of the ISR of each microbial species present in the soil. To prepare samples for fragment analysis, 2 µl of each PCR product were diluted with deionized water (to 10 µl), and 2 µl of these mixtures were aliquoted into the individual wells of a PCR plate. These samples were placed (uncovered) in a 37ºC oven and dried overnight (prior to being shipped), and 9 µl of GeneScan™ 1000 ROX® was used as a size standard for these PCR products.

Because the length of the ISR of each species (or species complex as in some bacteria) is unique, by estimating the relative abundance of each ISR found within a sample, one can determine the level of biodiversity of microbiota in the soils of cliff crevices. To determine the abundance of each ISR (i.e. microbial species), I analyzed the electropherograms (from the fragment analysis) in GENEIOUS PRIME®, which allowed me to estimate the relative abundances each species of fungi and bacteria in the soils. Only peaks with a fluorescence value over 150 units (for all dyes) were included in the analysis

(Ramette 2009), as well as only the ISRs larger than 150 bp, as the primers used in this study amplify ca. 150 bp outside the targeted ISR region (George, 2017). These values were then used to calculate the Simpson's Index of biodiversity (D_s; Brower and Zar 1984, George 2017) for both fungi and bacteria, which was calculated using the formula:

$$D_s = 1 - \sum(p_i)^2$$

where p_i represents the relative abundance of each molecular operational taxonomic unit (MOTUs), which is based on the total fluorescence values of all MOTU peaks in the sample of soil. These MOTUs are used as a proxy for the various microbial species found in the soils, as each MOTU represents a unique species, but the taxonomic identity of that species is unknow.

I chose the Simpson's Index because it is less sensitive to variations in sample size (Banna and Gardner 1996), and because it is influenced more by evenness than richness (George 2017). In addition, compared with other diversity indices, such as the Shannon Index, Simpson's Index is not only unbiased but also has the smallest standard deviation (Lande 1996). Pairwise comparisons of Simpson's biodiversity indexes between the soils collected from bat roosts and cliff crevices without bats were made using a *t*-test ($\alpha = 0.05$), and differences in species richness were measured using a Mann-Whitney U-test ($\alpha = 0.05$) for both fungi and bacteria independently.

Microscopy of Mesofauna

The soil left over in the Eppendorf® tubes after DNA-extraction was completed was examined for the presence of mesofauna using microscopy, and photos were taken of specimens found in the soils. Soil samples were emptied into individual Petri dishes and using a dissection microscope (30x) I scanned the entire dish of soil looking for deceased

organisms and biological debris (e.g. spider exuviae, insect limbs, plant seeds, etc.). I then identified all biological items to the lowest taxonomic level possible, which were then used to determine the biodiversity of mesofauna in the crevices of cliffs using the Simpson's Index. Finally, the values of mesofaunal biodiversity between crevices with and without bats was statistically compared with a *t*-test ($\alpha = 0.05$). After visual microscopy was complete, all soil samples were re-stored in their individual Eppendorf® tubes for subsequent nutrient analysis, which was done to examine the impact of bats on the influx of nitrogen and phosphorus into the crevices of cliff-face ecosystems.

Results

During summer 2016, I collected 52 samples of soil from cliff crevices with roosting bats (n = 26) and without roosting bats (n = 26). The DNA was successfully isolated from all 52 samples of soils, and all bacterial DNA amplified accurately during PCR (Fig. 47). In addition, all but one sample of fungal DNA amplified successfully, totaling 103 samples of DNA that could be assessed via fragment analysis (99% PCR efficacy). All 103 samples of amplified DNA sent to ASU were successfully measured during fragment analysis, and the corresponding electropherograms were manually scored in GENEIOUS PRIME to determine the number of ISR peaks (MOTUs; proxy for microbial species) as well as the peak fluorescence of each MOTU (i.e. relative abundance of that 'species'; Fig. 48).

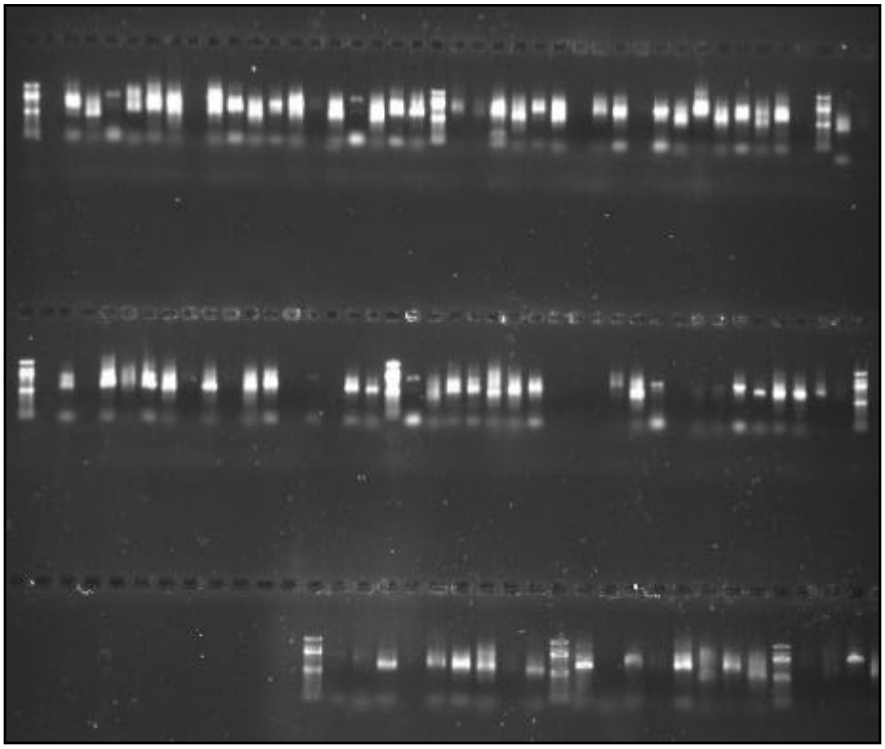

Fig. 47.—Agarose gel showing the results of PCR using the fluorescently labeled primers to amplify the ISRs of fungi and bacteria in the soils of cliff crevices.

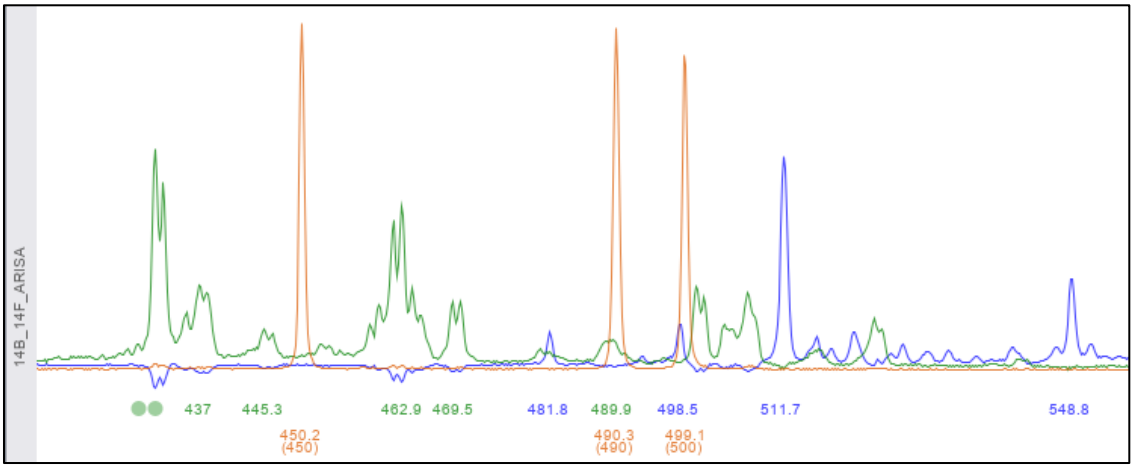

Fig. 48.— Electropherogram peaks from GENEIOUS PRIME of fungi (blue) and bacteria (green) with the size standard included (red). Numbers on the x-axis refer to the number of nucleotide bases in the ISR region of the organism.

By using ARISA, I amplified 294 MOTUs using bacterial primers, 219 of which were isolated from bat roosts and 113 that were found in crevices without bats. Of these MOTUs, only 38 were found in both types of crevices. The ISR length of bacteria found

within the soils of cliff crevices varied from 153 – 900 bp, with most of species having an ISR size of 250 – 880 bp.

Within an individual crevice the richness of bacteria ranged from one to 45 MOTUs, and on average the number of MOTUs present in soils was significantly higher in bat roosts (S = 13.7) relative to crevices without bats (S = 7.7; $U = 325$, $z = 2.32$, $p = 0.01$). There was a total of 12 bacterial MOTUs that were found in at least five different crevices, and two MOTUs (ISR lengths of 461 bp and 496 bp) were found in nine different crevices (Table 10). Moreover, I found nine MOTUs that were in at least three bat roosts but were not in a single cliff crevice without bats, indicating that there are species of bacteria present in bat roosts that are absent from crevices without bats (Table 11). Furthermore, based on the length of the ISR fragments, I was able to determine that six separate samples of soil contained the bacterial species *Lactobacillus brevis* (ISR length of 506 bp), a species known to occur in soils and that is isolated for its probiotic applications (Ghosh et al. 2015). Overall, the average Simpson's Index for the biodiversity of bacteria was significantly higher in bat roosts (0.83) than in crevices without bats (0.674; $t = 2.33$, $p = 0.012$).

Table 10.— Bacterial MOTUs (unique ISRs) found in at least five different samples of soil (10% of samples), showing the number of cliff crevices from which the MOTU was isolated.

ISR Length (bp)	Bat Roosts	Non-Bat Crevices
364	2	3
375	4	1
395	2	3
435	2	6
441	4	1
461	3	6
495	5	2
496	2	7
498	3	3
504	3	3
506	2	4
516	2	3

Table 11.—Bacterial MOTUs that were found in at least three different bat roosts, but not in a single cliff crevice without bats. Numbers represent the quantity of samples from which the MOTU was isolated based on its ISR length.

ISR Length (bp)	Bat Roosts	Non-Bat Crevices
311	3	0
352	3	0
384	3	0
389	3	0
393	4	0
436	4	0
509	4	0
607	3	0
621	4	0

With respect to the presence of soil fungi, I was able to amplify the DNA of 161 MOTUs from bat roosts and 196 MOTUs from non-roost crevices. Of these species, 88 MOTUs were found in both types of environments. The ISR lengths varied from 150 bp to 1,120 bp, with most of the species having an ISR size of 350 – 840 bp. The richness of

fungi within the soils of cliff crevices varied between 2 and 35 MOTUs, with the average number being slightly lower in crevices with bats (S = 10.7) than in crevices without bats (S = 14.9; $U = 349$, $z = 1.64$, $p = 0.054$). There were 23 MOTUs of fungi that were found in at least five samples of soil, with the ISR fragment of 529 bp being the most common species (which was isolated in 10 different cliff crevices; Table 12). In addition, there were three fungal MOTUs found in at least three bat roosts that were absent from cliff crevices without bats (ISR lengths of 527 bp, 535 bp, and 571 bp). Based on the length of the ISR regions amplified in the soil samples (Ghosh et al. 2015), I was able to isolate the fungi *Aureobasidium* sp. (1 sample), *Rhodotorula* sp. (2 samples), *Candida* sp. (4 samples), and *Cryptococcus* sp. (2 samples). The average Simpson's Index for the biodiversity of fungi was lower in bat roosts (0.721) than in crevices without bats (0.791), but this difference was only trending toward significance ($t = 1.62$, $p = 0.056$).

Table 12.—Unique MOTUs (fungal species) found in at least five different samples of soil (10% of samples). Below shows the number of cliff crevices from which each specific MOTU was isolated based on its ISR length.

ISR Length (bp)	Bat Roosts	Non-Bat Crevices
169	2	6
437	2	3
439	1	4
520	4	2
523	2	3
524	4	4
529	5	5
533	1	4
534	2	5
536	4	3
541	1	5
548	3	3
551	2	3
552	2	4
553	2	5
554	3	4
560	3	4
565	3	3
575	4	2
590	1	6
628	3	2
656	5	1
659	4	1

Microscopy of Mesofauna

With respect to the mesofauna within the soils of the cliff crevices on Dinosaur Mountain, I found relatively few biological specimens and little debris. Most crevice soils were without any mesofauna; however, some crevices (primarily those from bat roosts) contained dead millipedes, spider exuviae, pine seedlings, rootlets, mites, pieces of beetle exoskeleton, insect wings, and insect legs (Fig. 49). Overall, the Simpson's Index of biodiversity for mesofauna was higher in bat roosts (0.693) compared to crevices without bats (0.436; $t = 3.05$, $p = 0.0017$).

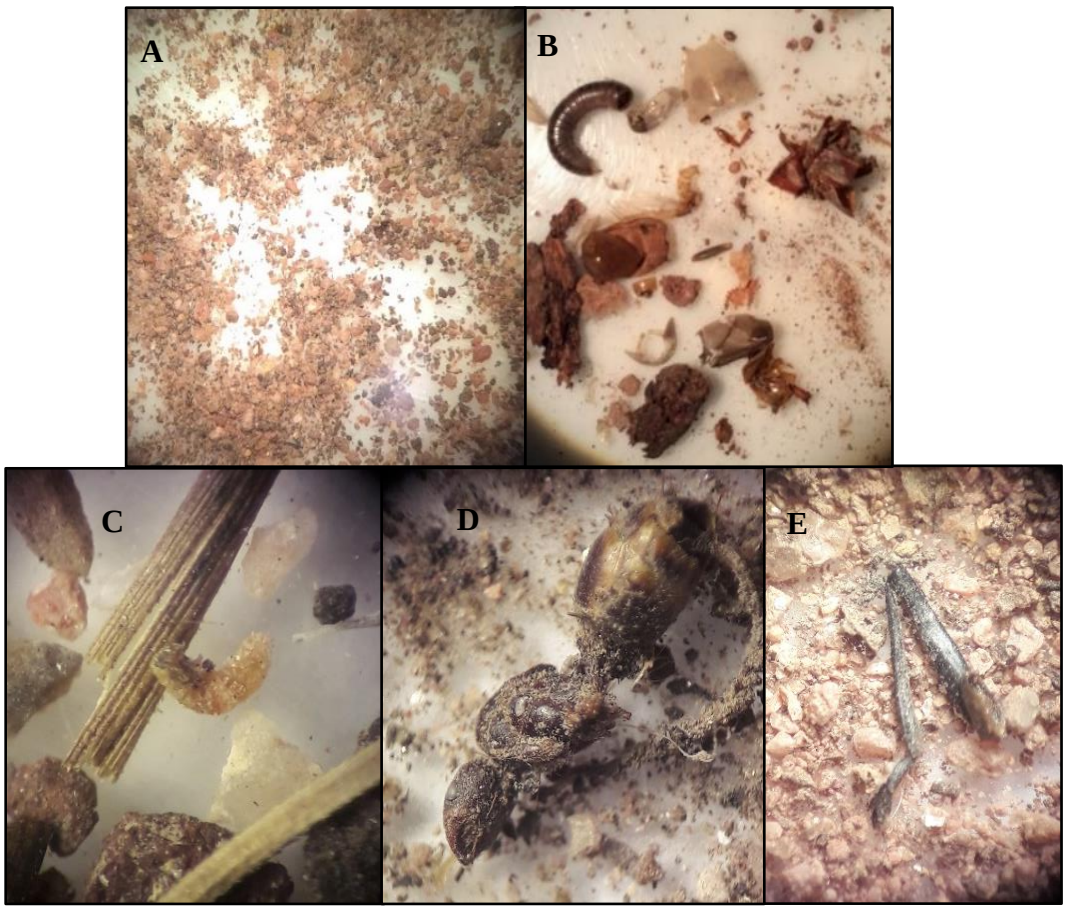

Fig. 49.—Mesofauna found within the soils of cliff crevices on Dinosaur Mountain. A) example of typical soils collected from crevices without bats, B) biological debris from a bat roost, C) beetle larva from bat roost, D) ant from bat roost, E) cricket leg from bat roost. Photos by A. K. Wilson.

Numerous living animals were observed on the vertical cliffs, talus slopes, and trails nearby rock-climbing sites. The most commonly seen invertebrate on the cliff crevices were wolf spiders (Family Lycosidae); however, I did not quantify presence/absence based on these observations during my study. The presence of these invertebrates was obvious when seeing the spiders scurry across the landscape, because the reflective tapetum lucidum (Benson and Suter 2013) of these nocturnal hunters exposed by our headlamps, indicated a vast prevalence across the faces of vertical cliffs. Other noteworthy invertebrates observed in these cliff crevices at every site include harvestmen (order Opiliones) and cave crickets (Rhaphidophoridae; Fig. 49). In addition,

the blue fungus beetles (*Gibbifer californicus*) was found at two sites (South Ridge and Dinosaur Rock, Fig. 50).

Fig. 50.—Invertebrates commonly seen on the cliffs of Dinosaur Mountain, A) cave crickets (Rhaphidophoridae), B) blue fungus beetles (*Gibbifer californicus*), C) Sun spider (Solifugae), D) female wolf spider (Lycosidae) with spiderlings on her abdomen, D) harvestmen (Opiliones).

With respect to the vertebrate species (other than bats) observed on the cliffs, numerous rock wrens (*Salpinctes obsoletus*) were heard throughout Dinosaur Mountain, but I was only able to observe one breeding pair within a cliff crevice on Red Devil (not pictured). I also saw American crows (*Corvus brachyrhynchos*) nesting on the flatirons (seen from the top of Front Porch), wild turkey (*Meleagris gallopavo*; Fig. 51) resting on the boulders of Dinosaur Rock, and poor-wills (*Phalaenoptilus nuttallii*) along the talus slopes and nearby trails. In addition, I observed deer mice (*Peromyscus maniculatus*; Fig. 51) nesting in the crevices on Der Freischutz, a chipmunk (*Tamias* sp.) peering from its home on Dinosaur Rock (Fig. 51), a striped skunk (*Mephitis mephitis*) that had made its den in the talus rocks of Front Porch, and a fence lizard (*Sceloporus undulatus*)

running across the talus slope of Bear Creek Spire. I also encountered a red fox (*Vulpes fulva*), black bear (*Ursus americanus*), prairie rattlesnake (*Crotalus viridis*), bullsnake (*Pituophis catenifer*; Fig. 51), and a milk snake (*Lampropeltis triangulum*) along the NCAR and/or Mesa trails that surround the cliffs of Dinosaur Mountain.

Fig. 51.—Some of the vertebrate species (other than bats) observed using the cliffs and nearby areas for various purposes. A) deer mouse (*Peromyscus maniculatus*), B) chipmunk (*Tamias* sp.), C) bullsnake (*Pituophis catenifer*), D) female wild turkey (*Meleagris gallopavo*).

Discussion

There is a direct link between biodiversity and ecosystem functions such as primary productivity, nutrient cycling, nitrogen fixation, carbon sequestration, etc. (Tilman et al. 2012; Pasari et al. 2013). Interestingly, Kolb et al. (2015) found that the guano deposited by cliff-dwelling birds influenced the biodiversity of microbiota and arthropods within the substrates of the crevices. Similarly, bats have been found to be important conduits of energy and nutrients in cave ecosystems (Studier et al. 1991; Iskali

and Zhang 2015), and the loss of bats from these caves negatively impacted by the biodiversity of the cave ecosystem (Hobbs and Bagley 1989). This study was the first to investigate how the presence of bats impacts the biodiversity of bacteria, fungi, and mesofauna within the crevices of vertical cliffs, where I hypothesized that the biodiversity of all three of the clades of organisms would be greater in bat roost than in crevices without bats.

Microbial Biodiversity of Cliff-Face Ecosystems

Although ARISA has been extensively used to analyze the DNA of soil microbiota (Ranjard et al. 2001; Okubo and Sugiyama 2009), it is still a novel method in the realm of bat biology. Recent work by George (2017) used ARISA to determine differences in the microbial communities between bat species, in addition to differences in the microbial communities on different regions of a bat's body. The bacterial diversity did vary among bat species, seasons, and body region, but the greatest differences in bacterial biodiversity was found among geographical regions (George 2017). Similarly, I found differences in the biodiversity of bacteria between areas, with the soils from bat roosts having a significantly higher biodiversity of bacteria than in cliff crevices without bats (Simpson's Index 0.83 and 0.67, respectively).

Previous studies have found a positive correlation between the biodiversity of soil bacteria and the primary productivity of the plants in the area (Laforest-Lapointe et al. 2017). Similarly, Delgado-Baquerizo et al. (2016) found that when bacterial biodiversity was decreased under controlled conditions, that the degradation of local toxins was significantly lowered (i.e. a reduction in ecosystem functionality). Furthermore, their study found no functional redundancy among the bacterial species, suggesting that a

lower biodiversity of bacteria results in a decrease in ecosystem functionality (Delgado-Baquerizo et al. 2016). Although my study did not investigate the ecosystem functionality of the bacteria living within the cliff crevices on Dinosaur Mountain, based on the findings of other studies involving the relationship between the biodiversity of soil bacteria and ecosystem functionality (Delgado-Baquerizo et al. 2016; Laforest-Lapointe et al. 2017), it is reasonable to presume that cliff soils with higher bacterial biodiversity would provide greater ecosystem services than the soils with lower bacterial biodiversity. For example, Jung et al. (2016) found that the abundance of genes related to the nitrogen cycle in soils was significantly reduced when the biodiversity of bacteria was decreased. This study found that the level of denitrification was impaired with lower levels of bacterial biodiversity (Jung et al. 2016), so the rate of nitrification is likely greater in the soils from bat roosts, where the bacterial biodiversity was significantly higher. This makes further sense when considering that my data show higher levels of nitrate in bat roosts, where there is also a higher biodiversity of bacteria (Chapter IV). However, this relationship is merely correlative.

Interestingly, there was no difference in the biodiversity of fungi between bat roosts and cliff crevices without bats (Simpson's Index values of 0.72 and 0.79, respectively). This may be due to the high degree of competition that is commonly seen among soil fungi, which can change the composition of fungal species living in/on a substrate (Hiscox et al. 2018). For example, the mycelia of many fungi alter the pH of the substrate, making it unsuitable for other species, and other fungi release volatile compounds that inhibit the growth and sporulation of competing fungi (Heilmann-Clausen and Boddy 2005; Hiscox et al. 2018). This high degree of competition among

fungi may play a role in the lack of difference in biodiversity between bat roosts and crevices without bats.

In addition to competition between different species of fungi, it has long been known that bacteria and fungi often have an antagonistic relationship, since the discovery of the antibiotic properties of *Penicillium*. Many species of bacteria release secondary metabolites that are essential for them to survive in their environments, but many species use these chemicals for competition at a distance (Stubbendieck and Straight 2016). Because bacteria and fungi often exist in the same environments, there is competition between these two types of organisms in nature for nutrients and space (Mille-Lindblom et al. 2006). Mille-Lindblom et al. (2006) even found a trade-off between fungal growth and tolerance toward bacteria, and that when fungi are well established, they often out-competed the bacteria and gained greater biomass. This high degree of competition could explain why the biodiversity patterns of bacteria and fungi in this study were opposite of one another. In addition to direct competition for resources, microbial breakdown of bat guano yields a more-acidic environment by producing strong acids including sulfuric phosphoric (Audra et al. 2019), which could make the environment less hospitable for certain species of fungi. This could explain why the biodiversity of fungi was lower in bat roosts, where the bacterial biodiversity was significantly higher; however, the pH of the soils was not examined in this study.

Another potential explanation for the lack of difference in fungal biodiversity between bat roosts and crevices without bats is that the biodiversity of fungi decreases with increased disturbance of the soil (Cho et al. 2017). Therefore, it is possible that when crawling through their roosts when entering and leaving, the bats may disturb the

soil to the point at which the biodiversity of the fungi is lessened. This could explain why the fungal biodiversity was slightly lower in bat roosts, despite the fact that both phosphorus and nitrogen levels were higher in bat roosts (two nutrients that are limiting for the growth of fungi). Nottingham et al. (2018) examined the rate of cellulose decomposition between bacteria and fungi in soils based on nutrient limitations and found nitrogen was limiting for both types of organisms, but that when phosphorus was added to the soils, the bacteria out-competed the fungi. These results corroborate the findings of this study, where bats increased the levels of phosphorus in the soils of cliff-crevices which housed greater biodiversity of bacteria, but lower biodiversity of fungi.

Unfortunately, without further investigation it will remain unclear which, if any, of these potential hypotheses explains why the biodiversity of fungi is lower in bat roosts. It is important to note that this study did not control for the presence and absence of bats within the crevices; therefore, the biodiversity of microbiota may be impacted by other factors such as the availability of light, temperature, and humidity of the cliff crevices. It is possible that bats are actively choosing cliff crevices that are inherently better habitats for the growth and development of bacteria; however, the fact that I gathered soils from bat roosts and non-roosts within 5 m of one another, in addition to selecting crevices of a similar size should help to minimize the impact of these extraneous variables. Furthermore, my results suggest that bat guano contains significant levels of nitrate and phosphate, which are limiting nutrients for the growth of bacteria and fungi (Nottingham et al. 2018), which provides support that the presence of bats impacts the biodiversity of bacteria in cliff crevices.

Biodiversity of Mesofauna in Cliff-Face Ecosystems

As predicted, the biodiversity of mesofauna was significantly higher in bat roosts than in crevices without bats. Although this result was expected, it is important to note that this study did not control for the presence of bats within the crevices of the cliffs. Therefore, the relationship between the presence of bats and the biodiversity of mesofauna is correlative, and future studies should be conducted to test this question in a more-controlled setting.

Conclusions

Overall, the biodiversity of bacteria and mesofauna was significantly higher in bat roosts relative to cliff crevices without bats. However, the biodiversity of fungi was slightly lower in bat roosts, which may be due to an antagonistic relationship between bacteria and fungi. Although this is the first study to investigate the impacts of bats on the biodiversity of cliff-dwelling animals, this is not the first project to find that bats provide critical ecosystem services for the habitats in which they live.

CHAPTER IV

BATS AS A CONDUIT OF NURTIENTS INTO CLIFF-FACE ECOSYSTEMS

Abstract

Vertical cliffs are rare ecosystems that are often limited in the nutrients required for the establishment of plants and microbiota. Bats are known to use the crevices of vertical cliffs for roosting, leaving behind guano and urine in the crevices while inside. Because previous studies found bat guano to contain nutrients that are essential for plants (e.g. nitrogen, phosphorus, calcium, magnesium), I hypothesized that soils from bat roosts would contain higher levels of the macronutrients nitrogen and phosphorus than soils collected from crevices without bats. Using water-quality testing kits, I quantified the amount of nitrate, nitrite, and phosphate in bat guano, soils from bat roosts, and soils from cliff crevices without bats. I found phosphate to be highest in bat guano, followed by bat roosts, then crevices without bats ($H_2 = 12.61$, $p = 0.0018$). With respect to nitrogen, nitrate was highest in bat roosts followed by cliff crevices without bats ($H_{2\_} = 7.82$, $p \leq 0.02$), and nitrite was not detected in bat guano, but was significantly higher in bat roosts than crevices without bats ($H_2 = 28.41$, $p \leq 0.0001$). It appears that bat guano is a source of phosphate in cliff crevices, and guano appears to have a positive effect on the availability of nitrate in these cracks. It is likely that the guano and urine deposited into cliff crevices by roosting bats increases the productivity of bacteria in the soil,

resulting in a greater production of nitrate (from nitrite) via nitrification. This increased level of nitrate in bat roosts may be essential for the establishment of plants in these crevices, as nitrate is the most usable form of nitrogen for flora.

Introduction

Many vertical cliffs are deprived of nutrients, which results in phenomena such as stunted growth in plants (Larson et al. 2000). Although rainwater brings nutrients into terrestrial ecosystems, most cliff crevices are sheltered from precipitation. Therefore, the feces deposited into the crevices of cliffs may be imperative for providing essential nutrients to the soils of cliff-face ecosystems, which are needed to sustain communities of plants and microorganisms (e.g. fungi and bacteria). Because cliff-roosting bats feed on the wing, their guano likely supplies crevices with nutrients that are otherwise absent from cliff ecosystems, which may be essential for the growth and development of bacteria, fungi, and plants in these (often) nutrient-deprived cracks.

The macronutrients nitrogen (a major component of chlorophyll, amino acids, and nucleic acids) and phosphorus (needed to make ATP, cell membranes, and nucleic acids) are often the most-limiting factors for the growth of plants (Jobbagy and Jackson 2001; Osman 2013). These elements have been detected in the guano of big brown bats (*Eptesicus fuscus*—Studier et al. 1991; Studier et al. 1994), a species that roosts in the rock-climbing cliffs in Boulder, Colorado (Chapter II). However, there has yet to be a study that compares levels of nutrients in different environments due to the presence of bats (i.e. bat guano).

Guano has been collected for the purposes of fertilizer since ca. 1840s (although bird guano was used much more than bat guano at first), and it is typically collected in

caves where large populations of bats reside that deposit significant amounts of guano into deep mounds (Ünal et al. 2018). Although the fertilizing benefits of bat guano have been long-appreciated, only recently has the impacts of bat guano on plants been analyzed. For example, Sridhar et al. (2006) found that applying the guano of *Hipposideros speoris* (an insectivorous species) to crops, increased crop yield, as well as the shoot length and nitrogen uptake by the plants. Similarly, Ünal et al. (2018) exposed lettuce plants to different levels of bat guano and found that this fecal material significantly increased the amount of nitrogen and phosphorus available to the plant. Another interesting study by Grafe et al. (2011) found that the guano provided by Hardwicke's wooly bat (*Kerivoula hardwickii*) to pitcher plants in southeast Asia increased the foliar nitrogen levels of these "poor insect traps", providing direct evidence that bat guano can increase the usable nitrogen for plants.

Previous studies have also found nitrogen and phosphorus in bat guano (Studier et al. 1994; Emerson and Roark 2006) among other minerals (e.g. Cl, Ca, Mg, Fe, Zn, Cu; Altintas et al. 2006; Audra et al. 2019). And both Studier et al. (1994) and Emerson and Roark (2006) found higher levels of nitrogen in the guano of insectivorous bats, which is the dietary niche all six species of cliff-roosting bat on Dinosaur Mountain. However, to my knowledge no previous study has yet investigated the amount of nitrogen and phosphorus in the guano of cliff-dwelling bats, nor the impact of this guano on the nutrient content of soils within the crevices of cliffs. For this study, I hypothesized that bat guano would increase levels of nitrate, nitrite, and phosphate in soils within cliff crevices.

Methods

To determine the impact of bats on the availability of nutrients in cliff crevices, I measured the abundance of the limiting macronutrients nitrogen and phosphorus in soil samples collected from cliff crevices with (n = 29) and without bats (n = 32), as well as in guano pellets (n = 36) collected from bat roosts. I determined levels of nitrogen and phosphorus in these samples using methods that could be easily replicated, in addition to providing a measurable, affordable, and portable way of calculating such values in the field with very few, light-weight supplies.

Nitrogen levels were determined using WaterWorks™ Water Quality Test Strips by Industrial Test Systems, Inc., which measures levels of nitrate and nitrite in aqueous solutions. I gathered ca. 0.15 ml of substrate from each sample of soil I collected from bat roosts and from cliff crevices without bats, and I submerged the individual samples in 1 ml of deionized water. Each solution was left to incubate at room temperature for 24 hrs., after which I inserted the water quality test strips into the supernatant, being careful not to disturb the settled debris at the bottom of the tube (Fig. 52). From there, I compared the color of the test strip to the reference colors on the manufacturer's kit, which I used to determine the ppm of nitrate and nitrite within the sample. In addition to all soil samples, I also measured every guano pellet I collected from the cliff crevices for their levels of nitrate and nitrite. I did this by submerging individual guano pellets (ca. same volume as 0.15 ml of soil) in 1 ml of deionized water in a 2-ml centrifuge tube, then gently macerating the pellet with a blunt prob (to loosen its compacted contents) before letting the solution incubate at room temperature for 24 hours. From there, I followed the

same methods for measuring the levels of nitrate and nitrite in the guano, as describe for the samples of soil described prior.

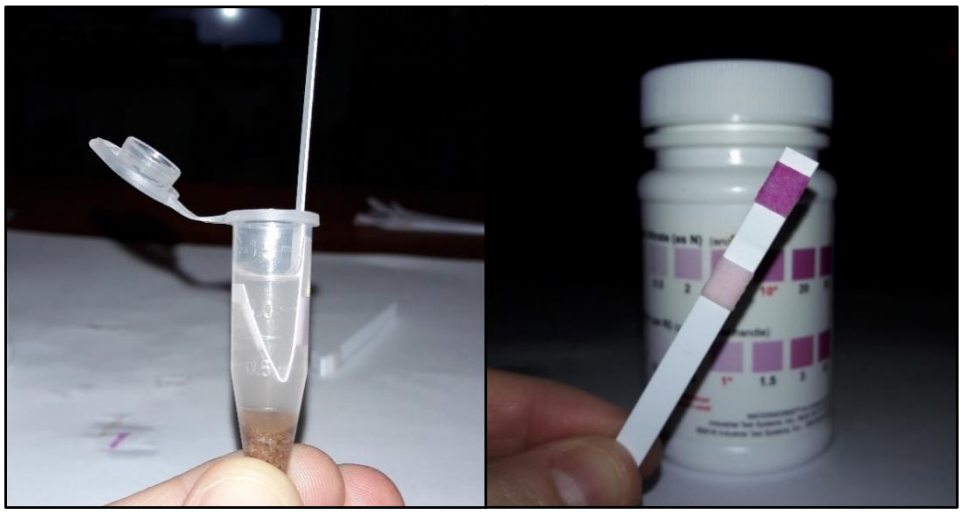

Fig. 52.—Methods for determining the levels of nitrogen (in the forms of nitrite and nitrate) within the soils from crevices with and without bats, as well as with guano found within the crevice (photos by A. K. Wilson).

To assess the levels of phosphorus (in the form of phosphate), I removed the supernatant (1 ml) from each sample of soil and guano (after measuring nitrogen levels) and placed it into the test tube provided with the PHOSPHATE test™ kit by Natural Chemistry L.P., which measures phosphate levels from 0 – 2,500 ppb. Following the manufacturer's protocol, I added deionized water to the individual supernatants (up to 10 ml) and placed a single test strip within the tube (containing the diluted supernatant; Fig. 53). I then tilted the tube (with the supernatant and test strip) back and forth three times, and I then compared the color of the solution to the reference chart provided with the manufacturer's kit to determine the ppb of phosphate within the sample. This process was repeated for each sample of soil and each guano pellet individually, and the test tube provided with the PHOSPHATE test™ kit was rinsed three times with deionized water in between samples.

The concentrations of nitrate, nitrite, and phosphate within the two types of soil samples, as well as within the bat guano, were statistically compared among each other using separate Kruskal-Wallis tests ($\alpha = 0.05$) for each ion under investigation. This test was used as the test stripes provide only categorical data whether the solution is over a certain level, but they do not indicate by how much the solution is over the categorical level (i.e. the measurements are not continuous).

Fig. 53.—Methods for determining phosphate levels within soils and bat guano (photos by A. K. Wilson).

Results

I measured levels of phosphorus and nitrogen from soil collected from cliff crevices with bats (n = 29) and from crevices without bats (n = 32), as well as from bat guano pellets (n = 36). Interestingly, bat guano had significantly higher levels of phosphate (513.9 ppb) relative to soils collected from bat roosts (268.9 ppb) and from crevices without roosting bats (118.8 ppb; $H_2 = 12.61$, $p = 0.0018$; Fig. 54). The fact that the amount of phosphate is highest within bat guano and lowest within soils from

crevices without bats, suggests that bat guano is a source of phosphate into the crevices of cliff-face ecosystems. Moreover, soils collected from bat roosts contained twice the phosphate as the soils collected from crevices without bats (Fig. 54), further suggesting that the fecal material deposited by bats while roosting increases the levels of phosphorus within the crevices of cliffs.

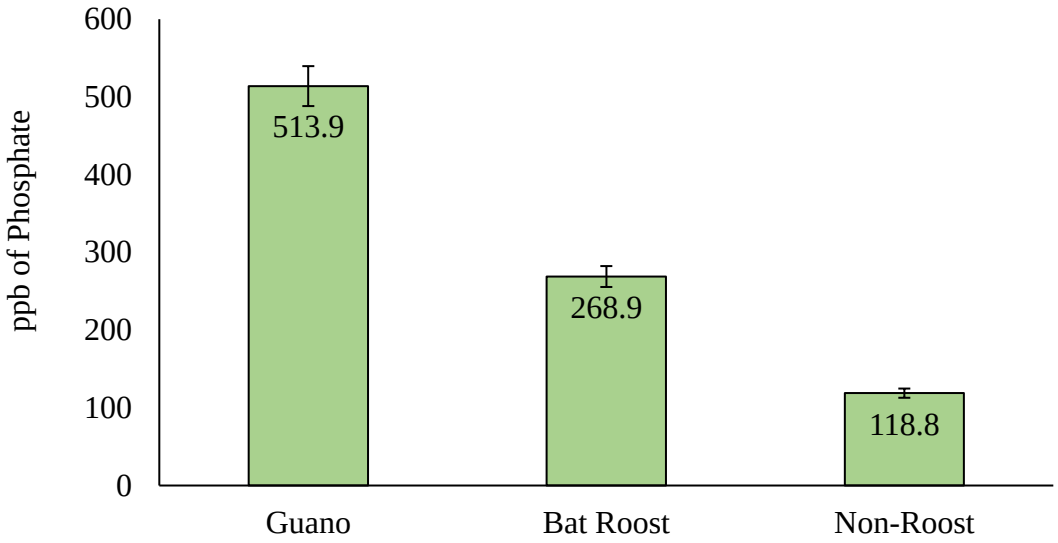

Fig. 54.—Mean levels of phosphorus, in the form of phosphate, within bat guano and soils collected from crevices with and without roosting bats (H_2 = 12.61; p = 0.0018).

Levels of nitrogen in the forms of nitrate and nitrite were both highest in the soils collected from bat roosts, followed by soils from crevices without bats, and lastly guano (Fig. 55; nitrate: H_2 = 7.82, $p \leq 0.02$; nitrite: H_2 = 28.41, $p \leq 0.0001$). Interestingly, not a single sample of guano contained detectable levels of nitrite, but nitrite levels were highest in soils from bat roosts, which tended to house a higher biodiversity of bacteria (which may be responsible for converting ammonium into nitrite, and nitrite into nitrate; Fig. 55). Nitrate levels were also lowest in bat guano (H_2 = 7.82, $p \leq 0.02$), although I was able to detect this ion in the feces of bats (Fig. 55).

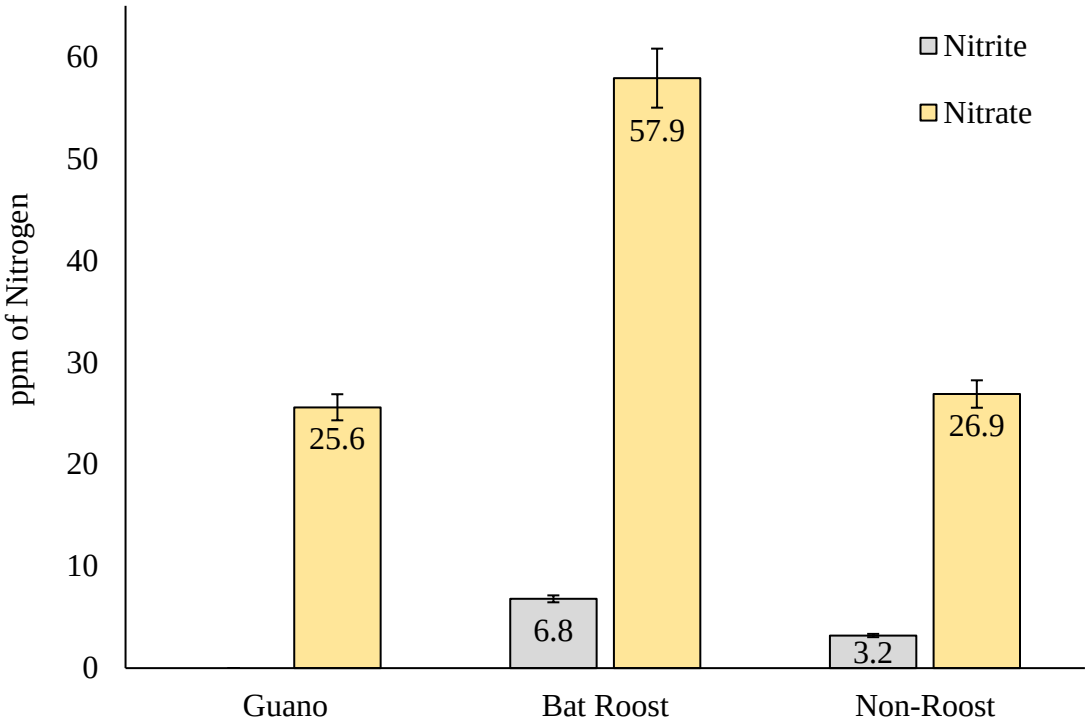

Fig. 55.—Mean levels of nitrogen, in the forms of nitrate (grey) and nitrite (orange), within bat guano and soils collected from crevices with and without roosting bats.

Discussion

Vertical cliffs are often deprived of nutrients, which can lead to in phenomena such as stunted growth in plants (Larson et al. 2000). By roosting in the crevices of cliff faces, bats likely provide essential nutrients that are needed to sustain communities of

plants and microorganisms (e.g. fungi and bacteria) by depositing guano and urine. I investigated the amount of nitrogen and phosphorus in bat guano, and soils collected from bat roosts and crevices without roosting bats.

By using methods that can easily be replicated in the field, the data I collected suggest that bats increase the levels of phosphate and nitrate in the soils of cliff crevices. Phosphate is the most common form of phosphorus found in nature, and it is an essential molecule for all living organisms as it is the backbone of DNA as well as a primary component of ATP. Phosphorus is not typically found in a gaseous form, but rather in rocks, soils, and other sediments, where is can be absorbed directly from the soil by plants (Audra et al. 2019). Therefore, the introduction of fast, usable phosphate from bat guano likely helps to provide this essential nutrient to the microbiota within the soils of cliff crevices, where nutrients are relatively rare.

Bat guano had nearly twice as much phosphate per unit as the soils from bat roosts (513.9 ppb and 268.9 ppb, respectively), which were both significantly higher than the level of phosphate found in the soils from cliff crevices without bats (Fig. 54). These results suggest to me that bat guano is a source of phosphate within cliff crevices. Unlike phosphorus, nitrogen is found in many forms in nature, and both mammalian urine and feces contain nitrogenous compounds (e.g. urea, uric acid, ammonia, etc.), which are converted to usable forms of nitrogen by soil bacteria. After urination and defecation, urea (when present) is converted to unstable ammonia (NH_3) by free-floating urease, which is quickly protonated and forms stable ammonium (NH_4). Bacteria in the soil continue to convert ammonium into nitrite (NO_2^-) and then nitrate (NO_3^-), which is the form of nitrogen that can be most easily be assimilated by plants. Mammals also excrete

some nitrate (and a bit of nitrite) as a waste product in their urine, feces, saliva, and sweat, which can be directly used by plants and soil microbiota without being converted by nitrifying bacteria first. Nitrate that is not assimilated by plants is further converted by denitrifying bacteria into atmospheric N_2 gas (where it must be fixed again before it can be used by plants and animals), or it may leech away as the negative charge of the ion will bind to water molecules brought in by the rain.

I was unable to find any detectable levels of nitrite within the guano pellets sampled; however, this ion was found in soil from cliff crevices both with and without bats at relatively low levels. Although it was not examined in this study, it would be interesting to see how levels of ammonium compared between these three groups. This would allow one to get a better understanding of whether the soil bacteria had converted all the ammonium and nitrite on the fecal pellets into nitrate, or whether nitrite levels within the waste of bats is truly negligible as it appears from my data.

Because mammalian wastes contain some nitrate, it is not surprising that levels of nitrate were highest in soils collected from bat roosts. Not only does the guano (and presumably urine) from roosting bats provide some nitrate to the soil, it is possible other nutrients supplied to the soils via guano (e.g. phosphate) could help to increase the number of nitrifying bacteria available in the soil, ultimately increasing levels of nitrate in the soils. This notion is further supported when considering that the bacterial biodiversity was higher in bat roosts (compared to non-roosts), which could further explain the higher levels of nitrate within bat roosts (i.e. more bacteria yields more nitrification). For example, Isbell et al. (2013) found a positive correlation between nitrogen levels in soil and the biodiversity of bacteria, so it is reasonable to presume that

increased nitrate available in bat roosts would help to increase the biodiversity of bacteria (or that the greater biodiversity of bacteria generates a larger amount of nitrate).

Interestingly, detectable levels of nitrate were found in the soils of cliff crevices without bats (mean 26.9 ppm) as well as in bat guano (mean 25.6 ppm), which when added together are still less than the average level of nitrate found in bat roosts (52.5 ppm vs. 57.9 ppm). These results suggest to me that there are other factors contributing to the addition of nitrate into the soils, which I believe is attributed to the higher biodiversity of bacterial life in the soils of bat roosts. Furthermore, when examining the difference in nitrite and nitrate levels within each type of soil, it is apparent that there is a greater discrepancy between these two ions within the soils of bat roosts, suggesting a greater degree of nitrification occurring within those soils (presumably due to a greater biodiversity of bacteria). Because nitrate is the form of nitrogen that is most easily assimilated by plants, it is promising that bats appear to provide nitrate within the cliff crevices, which could help plants to establish themselves within these crevices, increasing biodiversity of the entire cliff-face ecosystem.

Overall, my data suggest that bats impact the availability of essential macronutrients by introducing phosphate and nitrate into the crevices of cliff-face ecosystems. Both of these nutrients are critical for the development of bacteria, fungi, and plants, which are necessary for the stability of cliff-face ecosystems. The findings of this study are further corroborated by previous investigations, which found bat guano to increase levels of nitrogen and phosphorus available in the ecosystem (Altintas et al. 2005; Audra et al. 2019), which had positive impacts on the growth and availability of plants (Sridhar et al. 2006; Ünal et al. 2018). Although this study did not investigate the

impacts of the guano of cliff-dwelling bats on the growth and development of plants, the fact that the bat guano increased levels of phosphorus and nitrogen make it reasonable to assume that the feces of these bats would be beneficial for the plants of the Rocky-Mountain Foothills.

CHAPTER V

CONCLUSIONS

Many ecologists now accept that we are in the Anthropocene, the newest epoch of geological time where humans are the greatest forces of nature (Steffen et al. 2007). Human disturbances in this time range from increasing atmospheric CO_2 and radioactive nuclides, to urban sprawl, over-hunting, and invasive species (just to name a few). Rock-climbing is a relatively novel form of anthropogenic disturbance that has been shown to have negative impacts on the plants, lichens, and snails living on the faces of vertical cliffs. These rare ecosystems are capable of housing high biodiversity due to the heterogeneity of a cliff's substrate, aspect, grade, height, etc., which provides numerous microhabitats (in a small area) for supporting a variety of species with different physiological demands. Unfortunately, rock-climbing has been shown to decrease the biodiversity of cliff-dwelling flora, and the results of this study also suggest that this form of anthropogenic disturbance may negatively affect populations of cliff-roosting bats.

This dissertation was the first study to investigate the relationships between bats and cliff-face ecosystems. Specifically, I examined the impacts of rock climbing on the activity of bat in Boulder, Colorado (Chapter II), and found that increased rock-climbing resulted in lower bat richness and biodiversity, as well as a decrease in the number of bats roosting in the cliffs. Furthermore, when using Canonical Correspondence Analysis to correlate rock-climbing characteristics and bat activity, seasonal closures (to rock climbing) had a strong positive influence on bats on these cliffs. In addition, I quantified

the biodiversity of bacteria, fungi, and mesofauna living in the soils of cliff crevices (Chapter III), and I determined that the biodiversity of bacteria and mesofauna was significantly higher in bat roosts, whereas fungal biodiversity was slightly lower in these crevices. Lastly, I measured the amount of nitrogen and phosphorus in bat guano and soils from bat roost and cliff crevices without bats (Chapter IV) and found nitrate, nitrite, and phosphate all to be higher in bat roosts than crevices devoid of bats. Overall, this dissertation has shed light on the positive influence of bats on cliff-face ecosystems, as well as the negative impacts of rock climbing on these flying mammals.

Limitations of This Study

Of course, no scientific study is perfect, and all have their limitations and short-comings. There are some things that should be considered by future researchers when conducting studies similar to this one. For example, future studies should use multivariate statistics to examine correlations among rock-climbing characteristics and bat activity, making sure to include variables such as cliff angle and height, and average distance of roosts from climbing routes, which were not included in this study. If possible, future studies should also attempt to examine the internal conditions of crevice roosts, to get a better understanding of the physiological needs of these species during the summer, especially in maternity roosts. Future studies should also mist-net local bats and radio-track them to their roosts, which may be a more-feasible way of locating bat roosts on cliffs as opposed to years of visual observations. Unfortunately, in an attempt to minimize the amount of disturbance the bats experience on Dinosaur Mountain, I was not permitted to mist-net and radio-track bats on the property.

One the most-limiting aspects to my dissertation was not being allowed to observe the activity of bats on cliff sites when the sites were closed to rock climbers. Although seasonal closures are important and should be respected, it is difficult to assess the impact of rock climbing as a researcher when not allowed to access sites throughout the season. One example of how this convoluted this study is the fact that the only year I was able to find to direct evidence of a maternity roosts (i.e. seeing a neonate) was when I accessed Dinosaur Rock during its seasonal closure in 2014, before I realized I was not allowed access to that site during the closures. Since that first year, I obeyed all closures and was unable to find further direct evidence of maternity colonies, presumably due to always being at those sites late in the season when juveniles were weaned, and after the reintroduction of disturbance by the general public. In the future it would be important to monitor these locations both during and outside of seasonal closures, to get a better understanding of how these bats respond to anthropogenic disturbance. Therefore, if possible, future researchers may want to monitor such closed sites both during and outside of seasonal closures, to elucidate how human disturbances are impacting these bats.

Another major limitation to this study is the relatively uncontrolled means of testing the impacts of bats on the biodiversity of soils. Ideally, this aspect of the study would be stronger if I was able to prohibit bats from entering certain cliff crevices, which would act as control samples, while being able to access soils where large populations of bats constantly reside. For the location of my study sites, this method of a controlled study was not feasible, nor would I have wanted to restrict bats from potential maternity roost sites.

Management Implications and Future Directions

Because vertical cliffs are used by bats and humans, managers should try to conserve these mammals while still allowing the public to enjoy nature. However, it is essential that wildlife managers regulate the frequency of rock climbing on cliffs where threatened species are known to roost. For example, managers should also consider placing seasonal closures on cliffs where rare species of bat roost or where large maternity colonies are formed. Not only that, but managers could close only the routes on cliff-faces that pass near bat roosts, instead of restricting the entire cliff for the season. Wildlife managers at OSMP for example, should consider closing Front Porch (and possibly Lost Porch) from June through July due to the large richness and number of bats found roosting at this site. Moreover, the fact that relatively few people climb this site, closing Porch Alley (the trail that leads to Front Porch and Lost Porch) during these months will likely have little impact on the rock climbers that visit Dinosaur Mountain, but it could have a large influence on the local bats.

Other sites that I suggest being monitored more thoroughly for *M. thysanodes* include Bear Creek Spire and Dinosaur Rock, where this species was recorded. I recommend that OSMP hire rock climbers to investigate the roosts of these sites, to confirm the presence of the state-threated *M. thysanodes* at these cliffs. If this species is indeed roosting at Dinosaur Rock and Bear Creek Spire, it may require these rocks to be closed to anthropogenic disturbance during the summer months, when these bats are using the cliffs.

It may also be of interest to OSMP to continue monitoring the frequency of rock climbing at the rock-climbing sites on Dinosaur Mountain with time-lapse photography

throughout the year, to get an accurate assessment of the level of rock climbing at these sites. In addition, these cameras could also be used to determine whether people are respecting the seasonal closures, such as the one placed on the eastern face of Der Zerkle, where I found people returning from with my time-lapse photography. If there continues to be evidence of humans venturing to restricted parts of the Dinosaur Mountain, OSMP may want to consider roping-off closed areas more thoroughly, in addition to affixing more closure signs to the trees near these closed areas to help deter people from entering the restricted location.

Closing Remarks

Humans have an unmatched ability to alter their environment, often at the cost of the health of ecosystems. Previous research has shown that rock climbing has negative impacts on plants, lichens, and snails, and this dissertation has shed some light on the negative influences of this disturbance on the activity of cliff-roosting bats. Overall, the data from this study suggest that the richness and number of roosting bats, as well as the quantity of foraging bats were all greater in areas without rock climbing. The number of roosts, however, was greatest on sites with moderate rock climbing, which may be due to increased habitat heterogeneity (e.g. aspect, slope, substrate, etc.) of those sites relative to many of the unclimbed sites.

The bats on Dinosaur Mountain appear to influence cliff-face ecosystems positively by depositing guano into the crevices. Bat guano collected in cliff crevices was rich in phosphate and nitrate, which are limiting nutrients for bacteria, fungi, and plants. The availability of these nutrients was higher in the soils from bat roosts, and without these nutrients, there is no way for plants, herbivores, and higher-level organisms

to become established on these cliffs. This study also revealed that bat roosts contain greater biodiversity of bacteria, which are essential for the cycling of nitrogen and the decomposition of biological debris in every ecosystem. Therefore, frequent rock-climbing over long periods of time could negatively impact populations of bats on cliff-faces, which could ultimately have cascading effects on the availability of essential nutrients and bacterial biodiversity in the crevices of cliffs. Because of this, it is imperative that we properly manage anthropogenic disturbances (such as rock-climbing) on the faces of vertical cliffs before the biodiversity of these rare ecosystems dwindles and is lost forever.

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